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(FILE 'HOME' ENTERED AT 16:35:39 ON 03 DEC 1998)

FILE 'CAPLUS' ENTERED AT 16:35:49 ON 03 DEC 1998
L1 1033 S TELOMERASE
L2 692243 S BIND?
L3 110 S L1 AND L2
L4 951174 S HUMAN OR MOUSE OR MAMMAL###
L5 55 S L3 AND L4
L6 58064 S ASSOCIATE?
L7 27 S L6 AND L1 NOT L3
L8 24 S L4 AND L7
L9 827732 S INTERACT?
L10 28 S L1 AND L9 NOT (L2 OR L6)
L11 12 S L4 AND L10
L12 746004 S COMPLEX
L13 49 S L1 AND L12 NOT (L2 OR L6 OR L9)
L14 27 S L4 AND L13

FILE 'USPATFULL' ENTERED AT 16:51:53 ON 03 DEC 1998
L15 23 S L1(P)(L2 OR L6 OR L9 OR L12)(P)L4

L5 ANSWER 1 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Biological and molecular basis of *human* breast cancer

L5 ANSWER 2 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* as a novel target for anticancer therapy

L5 ANSWER 3 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI *Human* *Telomerase* Inhibition by Regioisomeric Disubstituted Amidoanthracene-9,10-diones

L5 ANSWER 4 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Purification of *human* *telomerase*

L5 ANSWER 5 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Inhibition of *human* *telomerase* by 2'-O-methyl-RNA

L5 ANSWER 6 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI An altered telomere repeat *binding* factor and its therapeutic use

L5 ANSWER 7 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI The implication of *telomerase* activity and telomere stability for replicative aging and cellular immortality (review)

L5 ANSWER 8 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Telomere elongation by hnRNP A1 and a derivative that interacts with telomeric repeats and *telomerase*

L5 ANSWER 9 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Porphyrin compounds as *telomerase* inhibitors

L5 ANSWER 10 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI NMR-Based Model of a *Telomerase* -Inhibiting Compound Bound to G-Quadruplex DNA

L5 ANSWER 11 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI The papillomavirus E6 proteins

L5 ANSWER 12 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Telomere length regulation in mice is linked to a novel chromosome locus

L5 ANSWER 13 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Advance of research on *telomerase*

L5 ANSWER 14 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Extra-chromosomal telomere repeat DNA in *telomerase* -negative immortalized cell lines

L5 ANSWER 15 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Tissue-specific and target RNA-specific ribozymes as antimicrobial therapeutics against microbial pathogens

L5 ANSWER 16 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* and chromosome end maintenance

L5 ANSWER 17 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Humanizing the yeast *telomerase* template

L5 ANSWER 18 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Dissociation of telomere dynamics from *telomerase* activity in *human* thyroid cancer cells

L5 ANSWER 19 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Species-specific and sequence-specific recognition of the dG-rich strand of telomeres by yeast *telomerase*

L5 ANSWER 20 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* -associated genes TCP2 and TCP3 and encoded protein and recombinant TCP2/TCP3-expressing cells

L5 ANSWER 21 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Cationic Porphyrins as *Telomerase* Inhibitors: the Interaction of Tetra(N-methyl-4-pyridyl)porphine with Quadruplex DNA

L5 ANSWER 22 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Preparation of *telomerase* from rat cell culture

L5 ANSWER 23 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Inhibition of *human* *telomerase* using peptide nucleic acids.

L5 ANSWER 24 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Association of nucleoside diphosphate kinase nm23-H2 with *human* telomeres

L5 ANSWER 25 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Reconstitution of *human* *telomerase* activity in vitro

L5 ANSWER 26 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Growth arrest of immortalized *human* keratinocytes and suppression of *telomerase* activity by p21WAF1 gene expression

L5 ANSWER 27 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Purification and recombinant production of *human* *telomerase* subunits and their applications for drug screening and therapy

L5 ANSWER 28 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Purification and recombinant production of *human* *telomerase* subunits and their applications for drug screening and therapy

L5 ANSWER 29 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* activity assays for diagnosing pathogenic infections

L5 ANSWER 30 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Abrogation of wild-type p53-mediated transactivation is insufficient for mutant p53-induced immortalization of normal *human* mammary epithelial cells

L5 ANSWER 31 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Characterization and cell cycle regulation of the related *human* telomeric proteins Pin2 and TRF1 suggest a role in mitosis

L5 ANSWER 32 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI identification of new step in telomere maintenance involving S-phase of cell cycle

L5 ANSWER 33 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Inhibitor peptide nucleic acids *binding* the RNA component of *mammalian* *telomerase*

L5 ANSWER 34 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI *Human* telomeres contain two distinct Myb-related proteins, TRF1 and TRF2

L5 ANSWER 35 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Characterization of *human* *telomerase* complex

L5 ANSWER 36 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Identification of Determinants for Inhibitor *Binding* within the RNA Active Site of *Human* *Telomerase* Using PNA Scanning

L5 ANSWER 37 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* and telomere- *binding* proteins: controlling the endgame

L5 ANSWER 38 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI A *mammalian* telomere repeat *binding* factor and the gene encoding it and their diagnostic and therapeutic uses

L5 ANSWER 39 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Regulation of telomere length and function by a Myb-domain protein in fission yeast

L5 ANSWER 40 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Control of telomere length by the *human* telomeric protein TRF1

L5 ANSWER 41 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI TRF1, a *mammalian* telomeric protein

L5 ANSWER 42 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Reconstitution of *human* *telomerase* activity and identification of a minimal functional region of the *human* *telomerase* RNA

L5 ANSWER 43 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI A review of the structure and function of *human* telomere

L5 ANSWER 44 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Telomere length regulation

L5 ANSWER 45 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI *Mammalian* *telomerase* RNA component sequences, regulation by oligonucleotides, and uses in gene therapy and cancer diagnosis

L5 ANSWER 46 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Immortalization of *human* mammary epithelial cells transfected with mutant p53 (273his)

L5 ANSWER 47 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Method for determining RNA 3' ends and application to *human* *telomerase* RNA

L5 ANSWER 48 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI A *human* telomeric protein

L5 ANSWER 49 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Preferential incorporation of 3'-Azido-2',3'-dideoxythymidine (AZT) in telomeric sequences of CHO cells

L5 ANSWER 50 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI The fidelity of *human* *telomerase*

L5 ANSWER 51 OF 55 CAPLUS COPYRIGHT 1998 ACS

TI Involvement of RB-1, p53, p16INK4 and *telomerase* in immortalization of *human* cells

L5 ANSWER 52 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Structure of the Drosophila HeT-A transposon: a retrotransposon-like element forming telomeres

L5 ANSWER 53 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI *Mammalian* telomere dynamics: healing, fragmentation shortening and stabilization

L5 ANSWER 54 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI Stringent sequence requirements for the formation of *human* telomeres

L5 ANSWER 55 OF 55 CAPLUS COPYRIGHT 1998 ACS
TI A yeast protein that *binds* to vertebrate telomeres and conserved yeast telomeric junctions

L5 ANSWER 2 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1998:719403 CAPLUS
TI *Telomerase* as a novel target for anticancer therapy
AU Hiyama, Keiko; Hiyama, Eiso
CS Sch. Med., Hiroshima Univ., Hiroshima, 734-8551, Japan
SO Mol. Med. (Tokyo) (1998), 35(11), 1374-1382 CODEN: MOLMEL; ISSN: 0918-6557
PB Nakayama Shoten DT Journal LA Japanese
AB A review with 28 refs. The nature of telomere and *telomerase* are described, and stem cells of the tissues with regeneration express *telomerase* in normal tissue. *Telomerase* is pos. in all kinds of tumors, and 10-20% of tumor samples are neg. for *telomerase* in almost all types of tumor. The regulation of *telomerase* activity in *human* cells are described. Suppression of *telomerase* is by induction of differentiation, suppression of *human* *telomerase* reverse transcriptase, and suppression of *human* *telomerase* RNA. The importance of *human* *telomerase* -assocd. protein 1 and TTAGGG repeat *binding* factors as the target of tumor therapy has not been elucidated. Delayed expression of the effects, effects for normal cells, and existence of *telomerase* -neg. tumor are to be considered. The elongation mechanism of telomere other than *telomerase* is suggested in vitro and in vivo tumor.

L5 ANSWER 6 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1998:568920 CAPLUS DN 129:199594
TI An altered telomere repeat *binding* factor and its therapeutic use
IN De Lange, Titia; Van Steensel, Bas; Bianchi, Alessandro
PA The Rockefeller University, USA
SO PCT Int. Appl., 164 pp. CODEN: PIXXD2 PI WO 9836066 A1 19980820
DS W: AU, CA, JP, MX
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 98-US2765 19980213 PRAI US 97-800264 19970213 DT Patent LA English
AB The present invention provides an isolated altered vertebrate telomere repeat *binding* factor (A-TRFs). Also included are the corresponding nucleic acids that encode the A-TRFs of the present invention, as well as the heterodimers formed by the assocn. of an A-TRF with a TRF. In addn., pharmaceutical compns. contg. the A-TRFs for treatment of diseases such as ataxia telangiectasia are also included. Methods of making, purifying and using the A-TRFs of the present invention are described. In addn., drug screening assays to identify drugs that mimic and/or complement the effect of the A-TRFs are presented. Long term overexpression of *human* TRF1 in the *telomerase* -pos. tumor cell line HT1080 resulted in a gradual and progressive telomere shortening. However, a dominant-neg. allele that inhibited *binding* of endogenous TRF1 to telomeres, induced telomere elongation. The dominant-neg. allele encoded a truncated TRF1 missing the DNA- *binding* domain, which hindered TRF1 *binding* to its DNA *binding* site, TTAGGG. Results obtained with a yeast two-hybrid assay in conjunction with in vitro DNA *binding* studies implicate the TRF-specific domain of TRF1 (residues 66-263) in dimerization. Overexpression in *human* fibrosarcoma cells of two deletion derivs. of *human* TRF2 lacking the basic N-terminus induced an irreversible growth arrest with characteristics of cellular senescence. This strong dominant-neg. allele caused loss of endogenous TRF2 from telomeres and induced end-to-end chromosome fusions detectable in metaphase and anaphase cells.

L5 ANSWER 7 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1998:566530 CAPLUS DN 129:300327
TI The implication of *telomerase* activity and telomere stability for replicative aging and cellular immortality (review)
AU Engelhardt, Monika; Martens, Uwe M.
CS Department of Hematology/Oncology, University of Freiburg, Freiburg, 79106, Germany
SO Oncol. Rep. (1998), 5(5), 1043-1052 CODEN: OCRPEW; ISSN: 1021-335X PB Oncology Reports
DT Journal; General Review LA English
AB A review with 109 refs. *Telomerase* activity and telomeres have been shown to be involved in the control of cell proliferation, the regulation of cell senescence, and the unlimited proliferation capacity of malignant cells. *Human* telomeres are specialized chromosomal end structures composed of TTAGGG repeats. They function to protect chromosomes from degrdn., fusion and recombination. Since the termini of linear mols. are replicated only in the 5'-3' direction by conventional DNA polymerases and require an RNA primer to initiate DNA synthesis, the removal of the RNA primer results in DNA loss with each cell division. To date, telomere shortening has een obsd. in most dividing somatic cells, eventually leading to cell senescence when critically short telomeres are reached. *Telomerase* has been identified as a ribonucleoprotein enzyme that can synthesize telomeric repeats onto chromosomes. Borderline *telomerase* activity has been detected in *human* primitive hematopoietic cells and in stimulated lymphocytes which increased with cytokine induced ex vivo expansion. However, in most other normal somatic cells, *telomerase* has not been detected, and consequently telomere shortening can be anticipated after a limited no. of population doublings. In contrast, spontaneously immortalized tumor cell lines and the majority of malignant tumors demonstrate high *telomerase* activity, stable telomere length, and unlimited proliferative potential. Mechanisms for *telomerase* and telomere length regulation are under extensive investigation. These have included the cloning of the RNA component and *telomerase* -assocd. proteins, antisense expts. that have demonstrated progressive telomere length shortening in the absence of *telomerase* , and the identification of telomere- *binding* proteins which may regulate *telomerase* by creating a neg. feedback signal. This review aims to summarize important results in the rapidly moving field of telomeres and *telomerase* .

L5 ANSWER 8 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1998:564971 CAPLUS DN 129:271447
TI Telomere elongation by hnRNP A1 and a derivative that interacts with telomeric repeats and *telomerase*
AU LaBranche, Helene; Dupuis, Sophie; Ben-David, Yaacov; Bani, Maria-Rosa; Wellinger, Raymund J.; Chabot, Benoit
CS Dep. Microbiologie Infectiologie, Fac. Med., Univ. Sherbrooke, Sherbrooke, PQ, J1H 5N4, Can.
SO Nat. Genet. (1998), 19(2), 199-202 CODEN: NGENEC; ISSN: 1061-4036 PB Nature America
DT Journal LA English
AB Telomeric DNA of *mammalian* chromosomes consists of several kilobase-pairs of tandemly repeated sequences with a terminal 3' overhang in single-stranded form. Maintaining the integrity of these repeats is essential for cell survival; telomere attrition is assocd. with chromosome instability and cell senescence, whereas stabilization of telomere length correlates with the immortalization of somatic cells. Telomere elongation is arried out by *telomerase* , an RNA-depend[redacted]A polymerase which adds single-stranded TAGGGT repeats to the 3' ends of chromosomes. While protei[redacted] assoc. with single-stranded telomeric repeats can influence tract lengths in yeast, equiv. factors have not yet been identified in vertebrates. Here, it is shown that the heterogeneous nuclear ribonucleoprotein A1 participates in telomere biogenesis. A *mouse* cell line deficient in A1 expression harbors telomeres that are shorter than those of a related cell line expressing normal levels of A1. Restoring A1 expression in A1-deficient cells increases telomere length. Telomere elongation is also obsd. upon introduction of exogenous UP1, the amino-terminal fragment of A1. While both A1 and UP1 *bind* to ertebrate single-stranded telomeric repeats directly and with specificity in vitro, only UP1 can recover *telomerase* activity from a cell lysate. These findings establish A1/UP1 as the first single-stranded DNA *binding* protein involved in *mammalian* telomere biogenesis and suggest possible mechanisms by which UP1 may modulate telomere length.

L5 ANSWER 11 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1998:497488 CAPLUS DN 129:299899
TI The papillomavirus E6 proteins
AU Rapp, Lisa; Chen, Jason J.
CS Department of Dermatology, New England Medical Center and Tufts University School of Medicine, Boston, MA, 02111, USA
SO Biochim. Biophys. Acta (1998), 1378(1), F1-F19 CODEN: BBACAQ; ISSN: 0006-3002
PB Elsevier Science B.V. DT Journal; General Review LA English
AB A review, with 209 refs. Specific types of *human* papillomaviruses (HPV) are strongly assocd. with the development of cervical cancer. The E6 gene from cancer-related HPVs has exhibited functions in tumorigenesis, regulation of transcription, *telomerase* , and apoptosis. Cancer-related HPVs E6 proteins *bind* the tumor suppressor p53 and promotes its degrdn. through an ubiquitin-dependent pathway. Several addnl. cellular E6- *binding* proteins have recently been identified and implicated in playing roles in p53-independent functions of E6.

L5 ANSWER 12 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1998:492612 CAPLUS DN 129:198825
TI Telomere length regulatis or *telomerase* components. These results demonstrate that an unidentified gene(s) mapped to distal chromosome 2 regulates telomere length in the *mouse* .

L5 ANSWER 13 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1998:480935 CAPLUS DN 129:241556
TI Advance of research on *telomerase*
AU Hiyama, Keiko; Ishioka, Shinichi; Hiyama, Eiso; Yamakido, Michio
CS Second Dep. Internal Medicine, School Medicine, Hiroshima Univ., Hiroshima, 734-8551, Japan
SO Gan to Kagaku Ryoho (1998), 25(8), 1105-1110 CODEN: GTKRDX; ISSN: 0385-0684
PB Gan to Kagaku Ryohosha DT Journal; General Review LA Japanese
AB A review with 21 refs. on *telomerase* , a ribonucleoprotein enzyme which can compensate for the erosion of telomeric repeats each cell division. The genes encoding the major components of *human* *telomerase* , TERC for *telomerase* RNA component, TEP1 for a *human* homolog of p80, and TERT for catalytic subunit, have been cloned. In addn., TERF1 and TERF2, encoding telomeric repeat *binding* proteins, have been cloned and shown to be involved in telomere length regulation and in the protection of chromosome end fusions, resp. Although the activity level of *telomerase* is similar at each stage of the cell cycle in *telomerase* -pos. cells, it is generally upregulated with cellular proliferation and repressed with withdrawal from the cell cycle. Although biol. mechanisms regulating *telomerase* activity and telomere length remain elucidated, *telomerase* activity can be detected in approx. 85% of *human* malignant neoplasms, and the *telomerase* is a promising and novel tumor marker for clin. diagnosis of cancer, as well as a novel target of anticancer therapy.

L5 ANSWER 20 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1998:183997 CAPLUS DN 128:240334
TI *Telomerase* -associated genes TCP2 and TCP3 and encoded protein and recombinant TCP2/TCP3-expressing cells
IN Villeponteau, Bryant; Feng, Junli; Andrews, William H.; Adams, Robert R.
PA Geron Corporation, USA
SO PCT Int. Appl., 86 pp. CODEN: PIXXD2
PI WO 9811204 A1 19980319
DS W: AU, CA, JP, MX
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 96-US14679 19960913 DT Patent LA English
AB Purified and recombinant proteins TPC2 and TPC3 and recombinant or synthetic oligonucleotides corresponding to gene TPC2 and TPC3 proteins or fragments may be used to detect regulators of telomere length and *telomerase* activity in *mammalian* cells and for a variety of related diagnostic and therapeutic purposes. Subtractive hybridization differential display was used to isolate and sequence *human* TPC2 and TPC3 gene protein cDNAs. TPC2 protein contains 2 WW domains and one L22 signature domain; TPC3 protein contains a homeobox domain. TPC2 protein may have a *telomerase* -sensing function; TPC3 protein may be a core component of the *telomerase* enzyme. TPC2 and TPC3 mRNA as well as the RNA component of *human* *telomerase* correlate with *telomerase* activity levels in a variety of mortal and immortal cell lines. TPC2 and TPC3 gene products can be used to diagnose cancer and other diseases. TPC3 mRNA in HeTe7 cells reduced *telomerase* activity and decreased telomere length.

L5 ANSWER 22 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1998:163674 CAPLUS DN 128:177567
TI Preparation of *telomerase* from rat cell culture
IN Yoshida, Shonen
PA Yoshida, Shonen, Japan
SO PCT Int. Appl., 20 pp. CODEN: PIXXD2 PI WO 9808938 A1 19980305
DS W: AL, AM, AU, AZ, BA, BB, BG, BR, BY, CA, CN, CU, CZ, EE, GE, GH, HU, IL, IS, KE, KG, KR, KZ, LC, LK, LR, LS, LT, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, RO, RU, SD, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI WO 97-JP2976 19970827 PRAI JP 96-226869 19960828 DT Patent LA Japanese
AB Proteins having a *telomerase* activity are prepd. from rat AH7974 cells by ammonium sulfate pptn., gel filtration using Sephacryl S-300 column, cation exchange chromatog. using Hyload SP column, and another gel filtration using Sephacryl S-400. The proteins catalyze the elongation of the telomere DNA 3'-OH terminal of chromosomes of eukaryotes; exhibit mol. wt. ranging from about 40 to about 120 kDa, in particular, about 110, 58 and/or 45 kDa, as detd. by SDS-PAGE.; inactivation. The proteins are inactivated by treating with RNase and able to *bind* to *mouse* *telomerase* RNA. The proteins are useful in screening *telomerase* inhibitors, developing diagnostic methods with the use of antibodies, etc.

L5 ANSWER 24 OF 55 CAPLUS COPYRIGHT 1998 ACS

AN 1998:123701 CAPLUS DN 128:253708
TI Association of nucleoside diphosphate kinase nm23-H2 with *human* te[illegible]s
AU Nosaka, Kazuto; Kawahara, Masahiro; Masuda, Mitsuharu; Satomi, Yos[illegible]shino, Hoyoku
CS Department of Biochemistry, Kyoto Prefectural University of Medicine, Kyo[illegible]602, Japan
SO Biochem. Biophys. Res. Commun. (1998), 243(2), 342-348 CODEN: BBRCA9; ISSN: 0006-291X
PB Academic Press DT Journal LA English
AB Telomeres, the ends of eukaryotic chromosomes, are essential structures formed by specific protein-DNA complexes that protect chromosomes from degrdn. and end-to-end fusion. TRF1, a double-stranded telomeric TTAGGG-repeat *binding* protein, is assocd. with *mammalian* telomeres and cont** *telomerase* .

L5 ANSWER 26 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1998:84977 CAPLUS DN 128:216039
TI Growth arrest of immortalized *human* keratinocytes and suppression of *telomerase* activity by p21WAF1 gene expression
AU Kallassy, Mireille; Martel, Nicole; Damour, Odile; Yamasaki, Hiroshi; Nakazawa, Hisayoshi
CS Unit of Multistage Carcinogenesis, International Agency for Research on Cancer, World Health Organization, Lyon, 69372, Fr.
SO Mol. Carcinog. (1998), 21(1), 26-36 CODEN: MOCAE8; ISSN: 0899-1987 PB Wiley-Liss, Inc.
DT Journal LA English
AB Because most non-melanocytic *human* skin cancers have p53 mutations, it is unclear whether the aberrant growth of these cancers is simply a result of the abrogation of a p53 downstream mediator, the universal cyclin-dependent kinase inhibitor p21WAF1. To investigate the role of p21WAF1 in *human* skin carcinogenesis, we studied its regulation in normal and p53-mutated immortalized *human* keratinocytes. In proliferating *human* normal keratinocytes (HNK), more wild-type p53 protein (wt p53) was expressed than in growth-arrested differentiating keratinocytes. However, the function of wt p53 as a transcriptional activator of the p21WAF1 gene was suppressed in proliferating keratinocytes. In response to UV B irradn., expression of wt p53 increased in proliferating keratinocytes, but p21WAF1 transcriptional activation was not induced. Two isoforms of mdm2 (p57 and p90), which can *bind* to wt p53 and neg. regulate wt p53 function, were expressed in proliferating HNK, suggesting that mdm2 may play a role in the suppression of wt p53's function in proliferating HNK. Increased expression of p21WAF1 was detected in both Ca2+-induced growth-arrested and differentiating HNK, in which the wt p53 expression was downregulated. This reflects the complexity of the p53/p21WAF1 pathways of cell-cycle regulation and differentiation in keratinocytes. No p21WAF1 expression was detected in (HaCaT) or in two ras-transformed variants, HaCaT ras 1/7 and HaCaT ras II/3, which have two p53 mutations. Retrovirus-mediated expression of p21WAF1 stopped the growth of all these cell types, but expression of wt p53 did not affect the cells' growth properties. P21WAF1 also downregulated *human* *telomerase* RNA component mRNA expression in HaCaT cells. This novel function of p21WAF1 partly explains the suppression of *telomerase* activity by p21WAF1 expression in HaCaT. These results are consistent with the idea that p21WAF1 successfully inhibits the growth of non-melanocytic skin cancers, even those with alterations in p53, p21ras, retinoblastoma gene product, and *telomerase* activity.

L5 ANSWER 27 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1998:65983 CAPLUS DN 128:150393
TI Purification and recombinant production of *human* *telomerase* subunits and their applications for drug screening and therapy
IN Cao, Zhaodan
PA Tularik, Inc., USA
SO PCT Int. Appl., 33 pp. CODEN: PIXXD2 PI WO 9801543 A1 19980115
DS W: AU, CA, JP
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 97-US12297 19970708 PRAI US 96-676967 19960708 DT Patent LA English
AB The invention provides methods and compns. relating to a *human* *telomerase* and related nucleic acids, including 4 distinct *human* *telomerase* subunit proteins called p140, p105, p48 and p43 having *human* *telomerase* -specific activity. *Human* *telomerase* p105 subunit cDNA contains an open reading frame encoding 759 amino acids. The proteins may be produced recombinantly from transformed host cellatent LA English
AB The invention provides methods and compns. relating to a *human* *telomerase* and related nucleic acids, including 4 distinct *human* *telomerase* subunit proteins called p140, p105, p48 and p43 having *human* *telomerase* -specific activity. *Human* *telomerase* p105 subunit cDNA contains an open reading frame encoding 759 amino acids. The proteins may be produced recombinantly from transformed host cells from the disclosed *telomerase* encoding nucleic acids or purified from *human* cells. Also included are *human* *telomerase* RNA components, as well as specific, functional derivs. thereof. The invention provides isolated *telomerase* hybridization probes and primers capable of specifically hybridizing with the disclosed *telomerase* gene, *telomerase* -specific *binding* agents such as specific antibodies, and methods of making and using the subject compns. in diagnosis, therapy and in the biopharmaceutical industry.

L5 ANSWER 31 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1997:802625 CAPLUS DN 128:139095
TI Characterization and cell cycle regulation of the related *human* telomeric proteins Pin2 and TRF1 suggest a role in mitosis
AU Shen, Minhui; Haggblom, Candy; Vogt, Marguerite; Hunter, Tony; Lu, Kun Ping
CS Cancer Biol. Program, Div. Hematol./Oncol., Dep. Med., Beth Israel Deaconess Med. Cent., Div. Aging, Harvard med. Sch., Boston, MA, 02215, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1997), 94(25), 13618-13623 CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences DT Journal LA English
AB Telomeres are essential for preserving chromosome integrity during the cell cycle and have been specifically implicated in mitotic progression, but little is known about the signaling mol.(s) involved. The *human* telomeric repeat *binding* factor protein (TRF1) is shown to be important in regulating telomere length. However, nothing is known about its function and regulation during the cell cycle. The sequence of PIN2, one of three *human* genes (PIN1-3) the authors previously cloned whose products interact with the Aspergillus NIMA cell cycle regulatory protein kinase, reveals that it encodes a protein that is identical in sequence to TRF1 apart from an internal deletion of 20 amino acids; Pin2 and TRF1 may be derived from the same gene, PIN2/TRF1. However, in the cell Pin2 was found to be the major expressed product and to form homo- and heterodimers with TRF1; both dimers were localized at telomeres. Pin2 directly bound the *human* telomeric repeat DNA in vitro, and was localized to all telomeres uniformly in *telomerase* -pos. cells. In contrast, in several cell lines that contain barely detectable *telomerase* activity, Pin2 was highly concd. at only a few telomeres. Interestingly, the protein level of Pin2 was highly regulated during the cell cycle, being strikingly increased in G2+M and decreased in G1 cells. Moreover, overexpression of Pin2 resulted in an accumulation of HeLa cells in G2+M. These results indicate that Pin2 is the major *human* telomeric protein and is highly regulated during the cell cycle, with a possible role in mitosis. The results also suggest that Pin2/TRF1 may connect mitotic control to the telomere regulatory machinery whose deregulation has been implicated in cancer and aging.

L5 ANSWER 32 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1997:740339 CAPLUS DN 128:976
TI identification of new step in telomere maintenance involving S-phase of cell cycle
IN Wellinger, Raymund J.; Zakian, Virginia A.
PA Trustees of Princeton University, U[illegible]llinger, Raymunctivities other than *telomerase* generate these TG1-3 tails and that their generation is a[illegible]gral step in telomere maintenance.

L5 ANSWER 37 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1997:468124 CAPLUS DN 127:201440
TI *Telomerase* and telomere- *binding* proteins: controlling the endgame
AU Shore, David
CS Dep. Molecular Biology, Univ. Geneva, Sciences II, Geneva, CH-1211, Switz.
SO Trends Biochem. Sci. (1997), 22(7), 233-235 CODEN: TBSCDB; ISSN: 0376-5067 PB Elsevier
DT Journal; General Review LA English
AB A review, with 30 refs. Topics discussed include: tetrahymena proteins; *human* , *mouse* and rat p80 homologues; *telomerase* catalysis in Euplotes and yeast; regulation of *telomerase* .

L5 ANSWER 38 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1997:267260 CAPLUS DN 126:247574
TI A *mammalian* telomere repeat *binding* factor and the gene encoding it and their diagnostic and therapeutic uses
IN De, Lange Titia
PA Rockefeller University, USA
SO PCT Int. Appl., 60 pp. CODEN: PIXXD2 PI WO 9708314 A2 19970306
DS W: CA, JP
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 96-US13652 19960823 PRAI US 95-519103 19950825 DT Patent LA English
AB A *mammalian* protein that *binds* the telomeric repeat TTAGGG is identified and a cDNA encoding it is cloned. The gene may be expressed in an appropriate hosts. In addn., antibodies, probes and antagonists specific for the telomeric protein are contemplated. Methods of identifying antagonists of the telomeric protein, diagnostic methods of identifying the telomeric protein in a sample, and therapeutic uses of the telomeric protein, particularly in the treatment of aging and cancer, are also contemplated. The protein was purified by affinity chromatog. against telomeric repeat-contg. DNA. Partial peptide sequences were used to design a hybridization probe that was used to screen a *human* HeLa cell library. Examn. of the sequence indicates that the protein has a Myb-type DNA- *binding* domain. Epitope-labeled protein manufd. in Escherichia coli was shown to *bind* to metaphase telomeres and the protein and telomeric DNA and the protein were colocated in vivo.

L5 ANSWER 40 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1997:145786 CAPLUS DN 126:247908
TI Control of telomere length by the *human* telomeric protein TRF1
AU van Steensel, Bas; de Lange, Titia
CS The Rockefeller Univ., New York, NY, 10021, USA
SO Nature (London) (1997), 385(6618), 740-743 CODEN: NATUAS; ISSN: 0028-0836
PB Macmillan Magazines DT Journal LA English
AB *Human* telomeres, the nucleoprotein complexes at chromosome ends, consist of tandem arrays of TTAGGG repeats bound to specific proteins. In normal *human* cells, telomeres shorten with successive cell divisions, probably due to the terminal sequence loss that accompanies DNA replication. In tumors and immortalized cells, this decline is halted through the activation of *telomerase* , a reverse transcriptase that extends the telomeric TTAGGG-repeat arrays. Telomere length is stable in several immortal *human* -cell lines, suggesting that a regulatory mechanism exists for limiting telomere elongation by *telomerase* . Here we show that the *human* telomeric-repeat *binding* factor TRF1 is involved in this regulation. Long-term overexpression of TRF1 in the *telomerase* -pos. tumor-cell line HT1080 resulted in a gradual and progressive telomere shortening. Conversely, telomere elongation was induced by expression of a dominant-neg. TRF1 mutant that inhibited *binding* of endogenous TRF1 to telomeres. Our results identify TRF1 as a suppressor of telomere elongation and indicate that TRF1 is involved in the neg. feedback mechanism that stabilizes telomere length. As TRF1 does not detectably affect the expression of *telomerase* , we propose that the *binding* of TRF1 controls telomere length in cis by inhibiting the action of *telomerase* at the ends of individual telomeres.

L5 ANSWER 42 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1996:702949 CAPLUS DN 126:86441
TI Reconstitution of *human* *telomerase* activity and identification of a minimal functional region of the *human* *telomerase* RNA
AU Autexi er, Chantal; Pruf chromosomes and species. Functions of telomere and *telomerase* in cell aging and tumorigenesis are discussed.

L5 ANSWER 45 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1996:171986 CAPLUS DN 124:222844
TI *Mammalian* *telomerase* RNA component sequences, regulation by oligonucleotides, and uses in gene therapy and cancer diagnosis
IN Andrews, William H.; Avillon, Ariel Athena; Feng, Junli; Funk, Walter; Greider, Carol; Marhuenda, Maria Antonia Blasco; Villeponteau, Bryant
PA Cold Spring Harbor Laboratory, USA; Geron Corporation
SO PCT Int. Appl., 84 pp. CODEN: PIXXD2 PI WO 9601614 A2 19960125
DS W: AM, AT, AU, BB, BG, BR, BY, CA, CH, CN, CZ, DE, DK, EE, ES, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LT, LU, LV, MD, MG, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, TJ, TM, TT
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI WO 95-US8620 19950707 PRAI US 94-272102 19940707 US 94-330123 19941027
US 95-387524 19950213 US 95-485778 19950607 DT Patent LA English
AB *Human* , *mouse* , rat, Chinese hamster, and cattle *telomerase* RNA component sequences were detd. Recombinant expression plasmids contg. *telomerase* RNA component genes are useful for gene therapy. Addnl., triple helix-forming oligonucleotides or antisense oligonucleotides are useful for regulating *telomerase* activity in vivo. Oligonucleotide probe *binding* by *telomerase* RNA components is also useful for cancer diagnosis.

L5 ANSWER 48 OF 55 CAPLUS COPYRIGHT 1998 ACS
AN 1995:984133 CAPLUS DN 124:79819
TI A *human* telomeric protein
AU Chong, Laura; van Steensel, Bas; Broccoli, Dominique;
Erdjument-Bromage, Hediye; Hanish, John; Tempst, Paul; de Lange, Titia
CS Lab. Cell Biol. Genet., Rockefeller Univ., New York, NY, 10021, USA
SO Science (Washington, D. C.) (1995), 270(5242), 1663-7 CODEN: SCIEAS; ISSN: 0036-8075
DT Journal LA English
AB Telomeres are multifunctional elements that shield chromosome ends from degrdn. and end-to-end fusions, prevent activation of DNA damage checkpoints, and modulate the maintenance of telomeric DNA by

telomerase . A major protein component of *human* telomeres has been identified and cloned. This factor, TRF, contains one Myb-type DNA- *binding* repeat and an amino-terminal [redacted] domain. Immunofluorescent labeling shows that TRF specifically colocalizes with telomeric DNA in *human* interphase cells and is located at chromosome ends during metaphase. The presence of TRF along the telomeric TTAGGG repeat array demonstrates that *human* telomeres form a specialized nucleoprotein complex.

L8 ANSWER 1 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Expression profile of senescence- *associated* b-galactosidase and activation of *telomerase* in *human* ovarian surface epithelial cells undergoing immortalization

L8 ANSWER 2 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* activity as a novel marker of lung cancer and immune- *associated* lung diseases

L8 ANSWER 3 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Oxyalkylene phosphate compounds and therapeutic uses thereof

L8 ANSWER 4 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Therapeutic augmentation of oxyalkylene diesters and butyric acid derivatives with inhibitors of fatty acid b-oxidation

L8 ANSWER 5 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Metabolically stabilized oxyalkylene esters and therapeutic uses thereof

L8 ANSWER 6 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Hydroxy- and ether-containing oxyalkylene esters and therapeutic uses thereof

L8 ANSWER 7 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Nitrogen-containing oxyalkylene esters and therapeutic uses thereof

L8 ANSWER 8 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Unsaturated oxyalkylene esters and therapeutic uses thereof

L8 ANSWER 9 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Transformation of primary *human* endothelial cells by Kaposi's sarcoma- *associated* herpesvirus

L8 ANSWER 10 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Immortalization of *human* oral keratinocytes is *associated* with elevation of *telomerase* activity and shortening of telomere length

L8 ANSWER 11 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Collagen disease- *associated* lung diseases and *telomerase* activity

L8 ANSWER 12 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Telomere and *telomerase* *associated* genes

L8 ANSWER 13 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Replicative senescence of normal *human* oral keratinocytes is *associated* with the loss of *telomerase* activity without shortening of telomeres

L8 ANSWER 14 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Gradual phenotypic conversion *associated* with immortalization of cultured *human* mammary epithelial cells

L8 ANSWER 15 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Downregulation of *telomerase* activity in HL60 cells by differentiating agents is accompanied by increased expression of *telomerase* - *associated* protein

L8 ANSWER 16 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Proliferation- *associated* regulation of *telomerase* activity in *human* endometrium and its potential implication in early cancer diagnosis

L8 ANSWER 17 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI A *mammalian* *telomerase* - *associated* protein

L8 ANSWER 18 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* activity is *associated* with cell cycle deregulation in *human* breast cancer

L8 ANSWER 19 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Evidence for UV- *associated* activation of *telomerase* in *human* skin

L8 ANSWER 20 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Telomere shortening is *associated* with cell division in vitro and in vivo

L8 ANSWER 21 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* activity *associated* with acquisition of malignancy in *human* colorectal cancer

L8 ANSWER 22 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Alterations in telomeric repeat length in lung cancer are *associated* with loss of heterozygosity in p53 and Rb

L8 ANSWER 23 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Telomere shortening *associated* with chromosome instability is arrested in immortal cells which express *telomerase* activity

L8 ANSWER 24 OF 24 CAPLUS COPYRIGHT 1998 ACS
TI Recognition of a chromosome truncation site *associated* with a-thalassaemia by *human* *telomerase*

L8 ANSWER 12 OF 24 CAPLUS COPYRIGHT 1998 ACS
AN 1998:56281 CAPLUS DN 128:111179
TI Telomere and *telomerase* *associated* genes
AU Tahara, Hidetoshi; Tahara, Eiji; Tahara, Eiichi; Ide, Toshinori
CS Dept. of Cellular and Mol. Biology, Dept. of Pathology, Hiroshima Univ. School of Medicine, Japan
SO Gan to Kagaku Ryoho (1997), 24(15), 2196-2201 CODEN: GTKRDX; ISSN: 0385-0684
PB Gan to Kagaku Ryohosha DT Journal; General Review LA Japanese
AB A review with 9 refs. *Telomerase* is a ribonucleoprotein, telomere specific reverse transcriptase, which contains some protein components and *telomerase* RNA components. *Human* *telomerase* RNA and some *telomerase* components have been identified but not completely. More recently, *human* *telomerase* catalytic subunits have been cloned, which are called hTRT or hEST2. The expression of hTRT in *human* cultured cells is well correlated with *telomerase* activity and immortality. Moreover, the expression of hTRT in cancer tissues is higher than that of normal tissues. These results suggested that hTRT and *telomerase* activity may be a powerful addnl. tool for cancer diagnosis.

L8 ANSWER 17 OF 24 CAPLUS COPYRIGHT 1998 ACS
AN 1997:124723 CAPLUS DN 126:222086
TI A *mammalian* *telomerase* - *associated* protein
AU Harrington, Lea; McPhail, Timothy; Mar, Vernon; Zhou, Wen; Oulton, Rena; Bass, Mike B.; Arruda, Isabel; Robinson, Murray O.
CS Amgen EST Program, Dep. Medical Biophysics, Univ. Toronto, Toronto, ON, M5G 2C1, Can.
SO Science (Washington, D. C.) (1997), 275(5302), 973-977 CODEN: SCIEAS; ISSN: 0036-8075
PB American Association for the Advancement of Science DT Journal LA English
AB The *telomerase* ribonucleoprotein catalyzes the addn. of new telomeres onto chromosome ends. A gene encoding a *mammalian* *telomerase* homolog called TP1 (*telomerase* -assocd. protein 1) was identified and cloned. TP1 exhibited extensive amino acid similarity to the Tetrahymena tolomerase protein p80 and was shown to interact specifically with *mammalian* *telomerase* RNA. Antiserum to TP1 immunopptd. *telomerase* activity from cell exts., suggesting that TP1 is assocd. with *telomerase* in vivo. The identification of TP1 suggests that *telomerase* -assocd. proteins are conserved from ciliates to humans.

L11 ANSWER 1 OF 12 CAPLUS COPYRIGHT 1998 ACS
TI Tricarboxylic acid-containing oxyalkyl esters, and therapeutic uses thereof

L11 ANSWER 2 OF 12 CAPLUS COPYRIGHT 1998 ACS
TI Impaired *telomerase* activity in uninfected hematopoietic progenitors in HIV-1-infected patients

L11 ANSWER 3 OF 12 CAPLUS COPYRIGHT 1998 ACS
TI Reconstitution of wild-type or mutant *telomerase* activity in *telomerase* -negative immortal *human* cells

L11 ANSWER 4 OF 12 CAPLUS COPYRIGHT 1998 ACS
TI *Human* gene encoding *telomerase* 2 and *human* and murine genes for *telomerase* RNA- *interacting* protein 1

L11 ANSWER 5 OF 12 CAPLUS COPYRIGHT 1998 ACS
TI Chromosomal instability is correlated with telomere erosion and inactivation of G2 checkpoint function in *human* fibroblasts expressing *human* papillomavirus type 16 E6 oncoprotein

L11 ANSWER 6 OF 12 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* from *human* leukemia cells: properties and its *interaction* with deoxynucleoside analoges

L11 ANSWER 7 OF 12 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* kinetics and mechanism: Using G-quadruplex *interactive* compounds as mechanistic probes.

L11 ANSWER 8 OF 12 CAPLUS COPYRIGHT 1998 ACS
TI Inhibition of *Human* *Telomerase* by a G-Quadruplex- *Interactive* Compound

L11 ANSWER 9 OF 12 CAPLUS COPYRIGHT 1998 ACS
TI Synthetic oligonucleotides which mimic telomeric sequences for use as neoplasm inhibitors

L11 ANSWER 10 OF 12 CAPLUS COPYRIGHT 1998 ACS
TI Peptide nucleic acids: characterization of their hybridization to duplex DNA and inhibitory effects on *human* *telomerase* activity (gene expression)

L11 ANSWER 11 OF 12 CAPLUS COPYRIGHT 1998 ACS
TI The *mouse* skin carcinogenesis model

L11 ANSWER 12 OF 12 CAPLUS COPYRIGHT 1998 ACS
TI Chromosome end associations, telomeres and *telomerase* activity in ataxia telangiectasia cells

L11 ANSWER 4 OF 12 CAPLUS COPYRIGHT 1998 ACS
AN 1998:344518 CAPLUS DN 129:50556
TI *Human* gene encoding *telomerase* 2 and *human* and murine genes for *telomerase* RNA- *interacting* protein 1
IN Harrington, Lea A.; Robinson, Murray O.
PA Amgen Inc., USA; Amgen Canada Inc.
SO PCT Int. Appl., 154 pp. CODEN: PIXXD2 PI WO 9821343 A1 19980522
DS W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: AT, BE, BF, BJ, CF, CG, CH, CI, CM, DE, DK, ES, FI, FR, GA, GB, GR, IE, IT, LU, MC, ML, MR, NE, NL, PT, SE, SN, TD, TG
AI WO 97-US21248 19971113 PRAI US 96-871189 19961115 US 97-873039 19970611 US 97-951733 19971016 DT Patent LA English
AB Disclosed are nucleic acid mols. encoding polypeptides of the *telomerase* complex. Specifically, cDNAs ere isolated encoding the *human* and murine *telomerase* 2 protein and the *human* *telomerase* RNA- *interacting* protein 1 (TRIP1), and their nucleotide and deduced amino acid sequences provided. Also disclosed are methods of prepg. the nucleic acid mols. and polypeptides, and methods of using these mols. These sequences may be used in methods for increasing cell proliferation or for increasing or decreasing the activity of *telomerase* .

L14 ANSWER 1 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Expression of the RNA component of *telomerase* during *human* development and differentiation

L14 ANSWER 2 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Synthetic 2'-O-methyl-modified hammerhead ribozymes targeted to the RNA component of *telomerase* as sequence-specific inhibitors of *telomerase* activity

L14 ANSWER 3 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Expression of *mouse* *telomerase* catalytic subunit in embryos and adult tissues

L14 ANSWER 4 OF 27 CAPLUS COPYRIGHT 1998 ACS

TI One-step affinity purification protocol for *human* *telomerase*

L14 ANSWER 5 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Targeted therapy of *human* malignant glioma in a *mouse* model by 2-5A antisense directed against *telomerase* RNA

L14 ANSWER 6 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Antigen-dependent regulation of *telomerase* activity in murine T cells

L14 ANSWER 7 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Reconstitution of *telomerase* activity in normal *human* cells leads to elongation of telomeres and extended replicative life span

L14 ANSWER 8 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Expression profile of the putative catalytic subunit of the *telomerase* gene

L14 ANSWER 9 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* activity in *human* leukemic cell lines is inhibited by antisense pentadecadeoxynucleotides targeted against c-myc mRNA

L14 ANSWER 10 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI *Human* *telomerase* contains evolutionarily conserved catalytic and structural subunits

L14 ANSWER 11 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Isolation of a candidate *human* *telomerase* catalytic subunit gene, which reveals *complex* splicing patterns in different cell types

L14 ANSWER 12 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Protein phosphatase 2A inhibits nuclear *telomerase* activity in *human* breast cancer cells

L14 ANSWER 13 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Bcl-2 modulates *telomerase* activity

L14 ANSWER 14 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* activity in normal *human* endothelial cells

L14 ANSWER 15 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* activity in *human* endometrium

L14 ANSWER 16 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* regulation during entry into the cell cycle in normal *human* T cells

L14 ANSWER 17 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* activity in oral leukoplakia and head and neck squamous cell carcinoma

L14 ANSWER 18 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* activity in the regenerative basal layer of the epidermis in *human* skin and in immortal and carcinoma-derived skin keratinocytes

L14 ANSWER 19 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Cell cycle-dependent modulation of *telomerase* activity in tumor cells

L14 ANSWER 20 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Characterization and expression of *human* *telomerase* (immortalization, replicative senescence)

L14 ANSWER 21 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI *Telomerase* activation by the E6 gene product of *human* papillomavirus type 16

L14 ANSWER 22 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Differentiation of immortal cells inhibits *telomerase* activity

L14 ANSWER 23 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Telomeres: beginning to understand the end

L14 ANSWER 24 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI The RNA component of *human* *telomerase*

L14 ANSWER 25 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Subtelomeric chromosome instability in Plasmodium falciparum: short telomere-like sequence motifs found frequently at healed chromosome breakpoints

L14 ANSWER 26 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Loss of telomeric DNA during aging may predispose cells to cancer

L14 ANSWER 27 OF 27 CAPLUS COPYRIGHT 1998 ACS
TI Repeated sequences organization in the telomeric regions of the eukaryotic genome

L14 ANSWER 1 OF 27 CAPLUS COPYRIGHT 1998 ACS
AN 1998:606675 CAPLUS
TI Expression of the RNA component of *telomerase* during *human* development and differentiation
AU Yashima, Kazuo; Maitra, Anirban; Rogers, Beverly Barton; Timmons, Charles F.; Rathi, Asha; Pinar, Halit; Wright, Woodring E.; Shay, Jerry W.; Gazdar, Adi F.
CS Hamon Center for Therapeutic Oncology Research, University of Texas Southwestern Medical Center, Dallas, TX, 75235, USA
SO Cell Growth Differ. (1998), 9(9), 805-813 CODEN: CGDIE7; ISSN: 1044-9523
PB American Association for Cancer Research DT Journal LA English
AB A radioactive in situ method was used to study the expression of the RNA component of *human* *telomerase* (hTR) during normal *human* development and differentiation using archival tissues. In embryonic tissues, the highest and most uniform expression was present in undifferentiated neuroepithelium. Expression was stronger in immature epithelium than in accompanying immature mesenchyme. Differentiation of most tissues was accompanied by decreased or absent expression. Except for the testis and adrenal gland, the adult pattern of expression was present by the 10th postnatal week. In adult tissues, high expression was present in the testis (primary spermatocytes and Sertoli cells), moderate expression was present in lymphoid follicles (germinal centers), and weak expression was present in epithelia (regenerative cells) but was absent in the nervous system and mesenchymal derived tissues. Expression in adult tissues was predominantly limited to dividing cells, although certain differentiated postmitotic cells expressed hTR. These studies demonstrate the *complex* interrelation of hTR expression [redacted] *human* development, differentiation, and cell division.

L14 ANSWER 3 OF 27 CAPLUS COPYRIGHT 1998 ACS
AN 1998:591349 CAPLUS DN 129:288173
TI Expression of *mouse* *telomerase* catalytic subunit in embryos and adult tissues
AU Martin-Rivera, Luis; Herrera, Eloisa; Albar, Juan P.; Blasco, Maria A.
CS Department of Immunology and Oncology, Centro Nacional de Biotecnologia, Consejo Superior de Investigaciones Cientificas, Madrid, 28049, Spain
SO Proc. Natl. Acad. Sci. U. S. A. (1998), 95(18), 10471-10476 CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences DT Journal LA English
AB *Telomerase* (I) is a ribonucleoprotein *complex* that elongates telomeres, allowing the stable maintenance of chromosomes during multiple cell divisions. Here, the authors describe the isolation and characterization of the catalytic subunit of *mouse* I, mTERT (*mouse* *telomerase* reverse transcriptase), an essential protein component of the I *complex* . During embryonic development, mTERT mRNA was abundantly expressed in the whole embryo, esp. in regions of intense proliferation. The authors found that mTERT mRNA expression in both embryonic and adult tissues was independent of the essential RNA component of I, mTR, and therefore, of the formation of active I complexes. The mTERT protein wass present exclusively in tissues with I activity, such as testis, spleen, and thymus. The mTERT protein is barely detectable in the thymus of mTR-/- mice, suggesting that mTERT protein stability in this tissue may depend on the actual assembly of active I complexes. Finally, it was found that *mouse* and *human* I catalytic subunit was located in the cell nucleus, and its localization was not regulated during cell cycle progression.

L14 ANSWER 4 OF 27 CAPLUS COPYRIGHT 1998 ACS
AN 1998:465169 CAPLUS DN 129:185906
TI One-step affinity purification protocol for *human* *telomerase*
AU Schnapp, Gisela; Rodi, Hans-Peter; Rettig, Wolfgang J.; Schnapp, Andreas; Damm, Klaus
CS Department of Oncology Research, Boehringer Ingelheim Pharma KG, Biberach an der Riss, 88397, Germany
SO Nucleic Acids Res. (1998), 26(13), 3311-3313 CODEN: NARHAD; ISSN: 0305-1048
PB Oxford University Press DT Journal LA English
AB *Human* *telomerase* is a ribonucleoprotein (RNP) enzyme, comprising protein components and an RNA template that catalyzes telomere elongation through the addn. of TTAGGG repeats. *Telomerase* function has been implicated in aging and cancer cell immortalization. We report a rapid and efficient one-step purifn. protocol to obtain highly active *telomerase* from *human* cells. The purifn. is based on affinity chromatog. Of nuclear exts. with antisense oligonucleotides complementary to the template region of the *human* telomerase* RNA component. Bound *telomerase* is eluted with a displacement oligonucleotide under mild conditions. The resulting affinity-purified *telomerase* is active in PCR-amplified *telomerase* assays. The purified *telomerase* *complex* has a mol. mass of .apprx.550 kDa compared to the .apprx.1000 kDa detd. for the *telomerase* RNP in unfractionated nuclear exts. The purifn. protocol provides a rapid and efficient tool for functional and structural studies of *human* *telomerase* .

L14 ANSWER 10 OF 27 CAPLUS COPYRIGHT 1998 ACS
AN 1998:6642 CAPLUS DN 128:124974
TI *Human* *telomerase* contains evolutionarily conserved catalytic and structural subunits
AU Harrington, Lea; Zhou, Wen; McPhail, Timothy; Oulton, Rena; Yeung, David S. K.; Mar, Vernon; Bass, Michael B.; Robinson, Murray O.
CS Department of Medical Biophysics, Amgen Institute/Ontario Cancer Institute, Department of Medical Biophysics, University of Toronto, Toronto, ON, M5G 2C1, Can.
SO Genes Dev. (1997), 11(23), 3109-3115 CODEN: GEDEEP; ISSN: 0890-9369
PB Cold Spring Harbor Laboratory Press DT Journal LA English
AB The authors have cloned and characterized a *human* gene encoding TP2 (*telomerase* -assocd. protein 2), a protein with similarity to reverse transcriptases and the catalytic *telomerase* subunits from Saccharomyces cerevisiae and Euplotes aediculatus. Indirect immunofluorescence revealed that TP2 was localized to the nucleus. Using antibodies to endogenous and epitope-tagged TP2, it was found that TP2 was assocd. Specifically with *human* *telomerase* activity and the recently identified *telomerase* -assocd. protein TP1. Mutation of conserved residues within the reverse transcriptase domain of TP2 severely reduced assocd. *telomerase* activity. These results suggest that *telomerase* is an evolutionarily conserved multisubunit *complex* composed of both structural and catalytic subunits.

L14 ANSWER 11 OF 27 CAPLUS COPYRIGHT 1998 ACS
AN 1997:711848 CAPLUS DN 128:44453
TI Isolation of a candidate *human* *telomerase* catalytic subunit gene, which reveals *complex* splicing patterns in different cell types
AU Kilian, Andrzej; Bowtell, David D. L.; Abud, Helen E.; Hime, Gary R.; Venter, Deon J.; Keese, Paul K.; Duncan, Emma L.; Reddel, Roger R.; Jefferson, Richard A.
CS CAMBIA, Canberra, ACT 2601, Australia
SO Hum. Mol. Genet. (1997), 6(12), 2011-2019 CODEN: HMGEE5; ISSN: 0964-6906
PB Oxford University Press DT Journal LA English
AB *Telomerase* is a multicomponent reverse transcriptase enzyme that adds DNA repeats to the ends of chromosomes using its RNA component as a template for synthesis. *Telomerase* activity is detected in the germline as well as the majority of tumors and immortal cell lines, and at low levels in several types of normal cells. We have cloned a *human* gene homologous to a protein from Saccharomyces cerevisiae and Euplotes aediculatus that has reverse transcriptase motifs and is thought to be the catalytic subunit of *telomerase* in those species. This gene is present in the *human* genome as a single copy sequence with a dominant transcript of -4 kb in a *human* colon cancer cell line, LIM1215. The cDNA sequence was detd. Using clones from a LIM1215 cDNA library and by RT-PCR, cRACE and 3'RACE on mRNA from the same source. We show that the gene is expressed in several normal tissues, *telomerase* -pos. post-crisis (immortal) cell lines and various tumors but is not expressed in the majority of normal tissues post-crisis (immortal) cell lines and various tumors but is not expressed in the majority of normal tissues analyzed, pre-crisis (non-immortal) cells and *telomerase* -neg. immortal (ALT) cell lines. Multiple products were identified by RT-PCR using primers within the reverse transcriptase domain. Sequencing of these products suggests that they arise by alternative splicing. Strikingly, various tumors, cell lines and even normal tissues (colonic crypt and testis) showed considerable differences in the splicing patterns. Alternative splicing of the *telomerase* catalytic subunit transcript may be important for the regulation of *telomerase* activity and may give rise to proteins with different biochem. functions.

L14 ANSWER 20 OF 27 CAPLUS COPYRIGHT 1998 ACS
AN 1996:199904 CAPLUS DN 124:257112
TI Characterization and expression of *human* *telomerase* (immortalization, replicative senescence)
AU Avilion, Ariel Athena
CS State Univ. of New York, Stony Brook, NY, USA
SO (1996) 235 pp. Avail.: Univ. Microfilms Int., Order No. DA9606364
From: Diss. Abstr. Int., B 1996, 56(11), 5930 DT Dissertation LA English AB Unavailable

L14 ANSWER 24 OF 27 CAPLUS COPYRIGHT 1998 ACS
AN 1995:783958 CAPLUS DN 123:329411
TI The RNA component of *human* *telomerase*
AU Feng, Junli; Funk, Walter D.; Wang, Sy-Shi; Weinrich, Scott L.; Avilion, Ariel A.; Chiu, Choy-Pik; Adams, Robert R.; Chang, Edwin; Allsopp, Richard C.; et al.
CS Geron Corp., Menlo Park, CA, 94025, USA
SO Science (Washington, D. C.) (1995), 269(5228), 1236-41 CODEN: SCIEAS; ISSN: 0036-8075
DT Journal LA English
AB Eukaryotic chromosomes are capped with repetitive telomere sequences that protect the ends from damage and rearrangements. Telomere repeats are synthesized by *telomerase*, a RNA (RNA)-protein *complex*. Here, the cloning of the RNA component of *human* *telomerase*, termed hTR, is described. The template region of hTR encompasses 11 nucleotides (5'-CUAACCCUAAC) complementary to the *human* telomere sequence (TTAGGG)n. Germline tissues and tumor cell lines expressed more Htr than normal somatic cells and tissues, which have no detectable *telomerase* activity. *Human* cell lines that expressed Htr mutated in the template region generated the predicted mutant *telomerase* activity. HeLa cells transfected with an antisense hTR lost telomeric DNA and began to die after 23 to 26 doublings. Thus, *human* *telomerase* is a crit. enzyme for the long-term proliferation of immortal tumor cells.

L15 ANSWER 1 OF 23 USPATFULL
AN 1998:147218 USPATFULL
TI Methods for diagnosis of conditions associated with elevated levels of telomerase activity
IN West, Michael D., Shay, Jerry, Dallas, Wright, Woodring
PI US 5840495 981124 AI US 95-480037 950607 (8)
RLI Division of Ser. No. US 93-38766, filed on 24 Mar 1993, now patented, Pat. No. US 5489508 which is a continuation-in-part of Ser. No. US 92-882438, filed on 13 May 1992, now abandoned

L15 ANSWER 2 OF 23 USPATFULL
AN 1998:147213 USPATFULL
TI Telomerase activity associated with hematological and colorectal malignancies
IN Bacchetti, Silvia, Counter, Christopher M., Leber, Brian, Harley, Calvin Bruce,
PI US 5840490 981124 AI US 95-485454 950607 (8)

L15 ANSWER 3 OF 23 USPATFULL
AN 1998:144253 USPATFULL
TI Mammalian telomerase
IN Villeponteau, Bryant, Feng, Junli, Funk, Walter, Andrews, William H.,
PI US 5837857 981117 AI US 96-660678 960605 (8)
RLI Continuation of Ser. No. US 94-330123, filed on 27 Oct 1994, now patented, Pat. No. US 5583016, issued on 10 Dec 1996 which is a continuation-in-part of Ser. No. US 94-272102, filed on 7 Jul 1994, now abandoned

L15 ANSWER 4 OF 23 USPATFULL
AN 1998:143858 USPATFULL
TI Telomerase activity assays
IN Harley, Calvin Bruce, Kim, Nam Woo, Weinrich, Scott Lawrence,
PI US 5837453 981117 AI US 95-482132 950607 (8)
RLI Continuation-in-part of Ser. No. US 94-315214, filed on 28 Sep 1994, now patented, Pat. No. US 5629154 which is a continuation-in-part of Ser. No. US 94-255774, filed on 7 Jun 1994 which is a continuation-in-part of Ser. No. US 93-153051, filed on 12 Nov 1993, now patented, Pat. No. US 5645987 And Ser. No. US 93-151477, filed on 12 Nov 1993, each Ser. No. US - which is a continuation-in-part of Ser. No. US 93-60952, filed on 13 May 1993, now patented, Pat. No. US 5695932 which is a continuation-in-part of Ser. No. US 93-38706, filed on 24 Mar 1993, now patented, Pat. No. US 5489508 which is a continuation-in-part of Ser. No. US 92-882438, filed on 13 May 1992, now abandoned

L15 ANSWER 5 OF 23 USPATFULL
AN 1998:138641 USPATFULL
TI Methods for measuring telomere length
IN Kozlowski, Michael R., Prowse, Karen R., Wang, Sy-Shi, Wong, Sharon, Kim, Nam Woo, Allsopp, Richard,
PI US 5834193 981110 AI US 96-660402 960607 (8)
RLI Continuation-in-part of Ser. No. US 95-479916, filed on 7 Jun 1995, now abandoned

L15 ANSWER 6 OF 23 USPATFULL
AN 1998:134800 USPATFULL
TI Method for screening for agents which increase telomerase activity in a cell
IN West, Michael D., Shay, Jerry, Wright, Woodring E.
PI US 5830644 981103 AI US 93-151477 931112 (8)
RLI Continuation-in-part of Ser. No. US 93-60952, filed on 13 May 1993 which is a continuation-in-part of Ser. No. US 93-38766, filed on 24 Mar 1993, now patented, Pat. No. US 5489508 which is a continuation-in-part of Ser. No. US 92-882438, filed on 13 May 1992, now abandoned

L15 ANSWER 7 OF 23 USPATFULL
AN 1998:108217 USPATFULL
TI Telomerase activity assays
IN Harley, Calvin Bruce, Kim, Nam Woo, Weinrich, Scott Lawrence
PI US 5804380 980908 AI US 96-632662 960415 (8)
RLI Continuation-in-part of Ser. No. US 95-482132, filed on 7 Jun 1995 which is a continuation-in-part of Ser. No. US 94-315214, filed on 28 Sep 1994, now patented, Pat. No. US 5629154 which is a continuation-in-part of Ser. No. US 94-255774, filed on 7 Jun 1994 which is a continuation-in-part of Ser. No. US 93-151477, filed on 12 Nov 1993 And Ser. No. US 93-153051, filed on 12 Nov 1993, now patented, Pat. No. US 5645986

L15 ANSWER 8 OF 23 USPATFULL
AN 1998:98932 USPATFULL
TI DHA-pharmaceutical agent conjugates of taxanes
IN Shashoua, Victor E., Swindell, Charles S., Webb, Nigel L., Bradley, Matthews O.
PI US 5795909 980818 AI US 96-651312 960522 (8)

L15 ANSWER 9 OF 23 USPATFULL
AN 1998:78929 USPATFULL
TI Assays for the DNA component of human telomerase
IN Villeponteau, Bryant, Feng, Junli, Funk, Walter, Andrews, William H.
PI US 5776679 980707 AI US 95-482115 950607 (8)
RLI Continuation-in-part of Ser. No. US 94-272102, filed on 7 Jul 1994, now abandoned And a continuation-in-part of Ser. No. US 94-330123, filed on 27 Oct 1994, now patented, Pat. No. US 5583016

L15 ANSWER 10 OF 23 USPATFULL
AN 1998:72454 USPATFULL
TI Human telomerase
IN Collins, Kathleen
PI US 5770422 980623 AI US 96-676974 960708 (8)

L15 ANSWER 11 OF 23 USPATFULL
AN 1998:48235 USPATFULL
TI *Human* *telomerase* RNA *interacting* protein gene
IN Cao, Zhaodan
PI US 5747317 980505 AI US 96-676967 960708 (8)

L15 ANSWER 12 OF 23 USPATFULL
AN 1998:33758 USPATFULL
TI Telomere repeat binding factor and diagnostic and therapeutic use thereof
IN De Lange, Titia
PI US 5733730 980331 AI US 95-519103 950825 (8)

L15 ANSWER 13 OF 23 USPATFULL
AN 1998:4398 USPATFULL
TI Therapy and diagnosis of conditions related to telomere length and/or telomerase activity
IN West, Michael D., Shay, Jerry, Wright, Woodring, Arlington
PI US 5707795 980113 AI US 95-487290 950607 (8)
RLI Division of Ser. No. US 93-38766, filed on 24 Mar 1993, now patented, Pat. No. US 5489508, issued on 6 Feb 1996 which is a continuation-in-part of Ser. No. US 92-882438, filed on 13 May 1992, now abandoned

L15 ANSWER 14 OF 23 USPATFULL
AN 97:118172 USPATFULL
TI Yeast telomerase compositions
IN Gottschling, Daniel E., Singer, Miriam S.,
PI US 5698686 971216 AI US 95-431080 950428 (8)
RLI Continuation-in-part of Ser. No. US 94-326781, filed on 20 Oct 1994, now abandoned

L15 ANSWER 15 OF 23 USPATFULL
AN 97:115098 USPATFULL
TI Telomerase activity assays for diagnosing pathogenic infections
IN West, Michael D., Shay, Jerry, Wright, Woodring, Blackburn, Elizabeth H., McEachern, Michael J.
PI US 5695932 971209 AI US 93-60952 930513 (8)
RLI Continuation-in-part of Ser. No. US 93-38766, filed on 24 Mar 1993, now patented, Pat. No. US 5489508 which is a continuation-in-part of Ser. No. US 92-882438, filed on 13 May 1992, now abandoned

L15 ANSWER 16 OF 23 USPATFULL
AN 97:112311 USPATFULL
TI Methods for cancer diagnosis and prognosis
IN Shay, Jerry, West, Michael David, Wright, Woodring E.
PI US 5693474 971202 AI US 95-486042 950607 (8)
RLI Continuation-in-part of Ser. No. US 95-423403, filed on 18 Apr 1995 which is a continuation-in-part of Ser. No. US 94-315216, filed on 28 Sep 1994 which is a continuation-in-part of Ser. No. US 94-255774, filed on 7 Jun 1994 which is a continuation-in-part of Ser. No. US 93-151477, filed on 12 Nov 1993 And Ser. No. US 93-153051, filed on 12 Nov 1993 which is a continuation-in-part of Ser. No. US 93-60952, filed on 13 May 1993 which is a continuation-in-part of Ser. No. US 93-38766, filed on 24 Mar 1993, now patented, Pat. No. US 5489508 which is a continuation-in-part of Ser. No. US 92-882438, filed on 13 May 1992, now abandoned, said Ser. No. US -151477 which is a continuation-in-part of Ser. No. US -60952

L15 ANSWER 17 OF 23 USPATFULL
AN 97:104277 USPATFULL
TI Methods for screening for agents which modulate telomere length
IN West, Michael D., Shay, Jerry, Wright, Woodring
PI US 5686245 971111 AI US 95-475778 950607 (8)
RLI Division of Ser. No. US 93-38766, filed on 24 Mar 1993, now patented, Pat. No. US 5489508 which is a continuation-in-part of Ser. No. US 92-882438, filed on 13 May 1992, now abandoned

L15 ANSWER 18 OF 23 USPATFULL
AN 97:59050 USPATFULL
TI Therapy and diagnosis of conditions related to telomere length and/or telomerase activity
IN West, Michael D., Harley, Calvin B., Strahl, Catherine M., McEachern, Michael J., Shay, Jerry, Wright, Woodring E., Blackburn, Elizabeth H., Vaziri, Homayoun,
PI US 5645986 970708 AI US 93-153051 931112 (8)
RLI Continuation-in-part of Ser. No. US 93-60952, filed on 13 May 1993 which is a continuation-in-part of Ser. No. US 93-38766, filed on 24 Mar 1993, now patented, Pat. No. US 5489508 which is a continuation-in-part of Ser. No. US 92-882438, filed on 13 May 1992, now abandoned

L15 ANSWER 19 OF 23 USPATFULL
AN 97:56656 USPATFULL
TI Synthetic oligonucleotides which mimic telomeric sequences for use in treatment of cancer and other diseases
IN Iversen, Patrick L., Mata, John E.
PI US 5643890 970701 AI US 95-381097 950131 (8)

L15 ANSWER 20 OF 23 USPATFULL
AN 97:51866 USPATFULL
TI Methods for cancer diagnosis and prognosis
IN Shay, Jerry, West, Michael David, Wright, Woodring E.,
PI US 5639613 970617 AI US 95-423403 950418 (8)
RLI Continuation-in-part of Ser. No. US 94-315216, filed on 28 Sep 1994 which is a continuation-in-part of Ser. No. US 94-255774, filed on 7 Jun 1994 which is a continuation-in-part of Ser. No. US 93-151477, filed on 12 Nov 1993 And a continuation-in-part of Ser. No. US 93-153051, filed on 12 Nov 1993 which is a continuation-in-part of Ser. No. US 93-60952, filed on 13 May 1993 which is a continuation-in-part of Ser. No. US 93-38766, filed on 24 Mar 1993, now patented, Pat. No. US 5489508 which is a continuation-in-part of Ser. No. US 92-882438, filed on 13 May 1992, now abandoned, said Ser. No. US -151477 which is a continuation-in-part of Ser. No. US 93-60952, filed on 13 May 1993

L15 ANSWER 21 OF 23 USPATFULL
AN 97:40634 USPATFULL
TI Telomerase activity assays
IN Kim, Nam W., Harley, Calvin B., Weinrich, Scott L.

PI US 5629154 970513 AI US 94-315214 940928 (8)
RLI Continuation-in-part of Ser. No. US 94-255774, filed on 7 Jun 1994 which [redacted]ntinuation-in-part of Ser. No. US 93-151477, filed on 12 Nov 1993 which is a continuation-in-part of Ser. No. US 93-153051, filed on 12 Nov 1993

L15 ANSWER 22 OF 23 USPATFULL
AN 96:113813 USPATFULL
TI Mammalian telomerase
IN Villeponteau, Bryant, Feng, Junli, Funk, Walter, Andrews, William H.
PI US 5583016 961210 AI US 94-330123 941027 (8)
RLI Continuation-in-part of Ser. No. US 94-272102, filed on 7 Jul 1994, now abandoned

L15 ANSWER 23 OF 23 USPATFULL
AN 96:11055 USPATFULL
TI Therapy and diagnosis of conditions related to telomere length and/or telomerase activity
IN West, Michael D. Shay, Jerry, Wright, Woodring
PI US 5489508 960206 AI US 93-38766 930324 (8)
RLI Continuation-in-part of Ser. No. US 92-882438, filed on 13 May 1992, now abandoned

L15 ANSWER 1 OF 23 USPATFULL
SUMM It has been known for some years that telomeres in *human* germline cells (e.g. sperm) are longer than those in somatic tissue such as blood. One proposed explanation for this is the absence of telomere repeat addition (i.e. absence of *telomerase* activity) in somatic cells. If so, incomplete end replication would be expected to result in the progressive loss of terminal. . . contribute to the chromosome aberrations typically seen in senescent cells. Senescence and the measurement of cellular time is an intriguingly *complex* subject and it will be interesting to see to what extent telomere shortening has a causal role. The large telomeres. . .
SUMM . . . special structure and behavior of telomeric DNA, suggest that telomere synthesis could be a target for selective drug action. Because *telomerase* activity seems to be essential for protozoans or yeast, but not apparently for *mammalian* somatic cells, I propose that *telomerase* should be explored as a target for drugs against eukaryotic pathogenic or parasitic microorganisms, such as parasitic protozoans or pathogenic yeasts. A drug that *binds* *telomerase* selectively, either through its reverse-transcriptase or DNA substrate- *binding* properties, should selectively act against prolonged maintenance of the dividing lower eukaryote, but not impair the *mammalian* host over the short term, because *telomerase* activity in its somatic cells may normally be low or absent. Obvious classes of drugs to investigate are those directed specifically against reverse transcriptases as opposed to other DNA or RNA polymerases, and drugs that would *bind* telomeric DNA itself. These could include drugs that selectively *bind* the G--G base-paired forms of the G-rich strand protrusions at the chromosome termini, or agents which stabilize an inappropriate G--G. . .
DETD As noted above, the present invention concerns diagnosis and therapy *associated* with measuring telomeric length and manipulating *telomerase* -dependent extension or *telomerase* -independent shortening. While the invention is directed to humans, it may be applied to other animals, particularly *mammals* , such as other primates, and domestic animals, such as equine, bovine, ovine, porcine, feline, and canine. The invention may be. . . may be slowed or inhibited by providing DNA oligonucleotides or their functional equivalent, or self-proliferation can be reduced by inhibiting *telomerase* . In this case of diagnostics, one may detect the length of telomeres as to a particular chromosome or group of chromosomes, or the average length of telomeres. Diagnosis may also be *associated* with determining the activity of *telomerase* in cells, tissue, and the like.
DETD *Telomerase* activity is of interest as a marker of growth potential, particularly as to neoplastic cells, or progenitor cells, e.g., embryonic cells. *Human* *telomerase* activity may be determined by measuring the rate of elongation of an appropriate repetitive sequence (primer), having 2 or more, usually 3 or more, repeats of the telomere unit sequence, TTAGGG. The sequence is labeled with a specific *binding* pair member at a convenient site, e.g., the 5'-terminus, which specific *binding* pair member allows for separation of extended sequences. By using one or more radioactive nucleotide triphosphates or other labeled nucleotide. . . incorporated radioactivity as cpm per unit weight of DNA as a function of unit of time, as a measure of *telomerase* activity. Any other detectable signal and label may also be used, e.g., fluorescein.
CLM What is claimed is:
1. A method for diagnosis in a patient of a condition *associated* with an elevated level of *human* *telomerase* activity within one or more cells, the method comprising the steps of: determining the presence or amount of *human* *telomerase* within said cells; and correlating said presence or amount of *telomerase* with a condition *associated* with an elevated level of *telomerase* activity.

27. A method for diagnosis in a patient of a condition *associated* with an elevated level of *mammalian* *telomerase* activity within one or more cells, the method comprising the steps of: determining the presence or amount of *mammalian* *telomerase* within said cells; and correlating said presence or amount of telomerase* with a condition *associated* with an elevated level of *telomerase* activity.

L15 ANSWER 2 OF 23 USPATFULL
DETD *Telomerase* Activity *Associated* with Acquisition of Malignancy in *Human* Colorectal Cancer

L15 ANSWER 3 OF 23 USPATFULL
SUMM . . . first aspect, the present invention provides the RNA component of, as well as the gene for the RNA component of, *human* *telomerase* in substantially pure form, as well as nucleic acids comprising all or at least a useful portion of the nucleotide sequence of the RNA component of *human* *telomerase* . The present invention also provides RNA component nucleic acids from other species, which nucleic acids share substantial homology with the RNA component of *human* *telomerase* , including but not limited to, the RNA components of *mammals* , such as primates. Other useful nucleic acids of the invention include nucleic acids with sequences complementary to the RNA component; nucleic acids with sequences related to but distinct from nucleotide sequences of the RNA component and which *interact* with the RNA component or the gene for the RNA component or the protein components of *human* *telomerase* in a useful way; and nucleic acids that do not share significant sequence homology or complementarity to the RNA component. . .
SUMM . . . triple helix-forming oligonucleotide, or other oligonucleotide that can be used in vivo or in vitro to inhibit the activity of *human* *telomerase* . Such oligonucleotides can block *telomerase* activity in a number of ways, including by preventing transcription of the *telomerase* gene (for instance, by triple helix formation) or by *binding* to the RNA component of *telomerase* in a manner that prevents a functional ibonucleoprotein *telomerase* from assembling or prevents the RNA component, once assembled into the *telomerase* enzyme *complex* , from serving as a template for telomeric DNA synthesis. Typically, and depending on mode of action, these oligonucleotides of the. . . or more nucleotides that is either identical or complementary to a specific sequence of nucleotides in the RNA component of *telomerase* or the gene for the RNA component of *telomerase* .
SUMM Another type of useful nucleic acid of the invention is a ribozyme able to cleave specifically the RNA component of *human* *telomerase* , rendering the enzyme inactive. Yet another type of useful nucleic acid of the invention is a probe or primer that *binds* specifically to the RNA component of *human* *telomerase* and so can be used, e.g., to detect the presence of *telomerase* in a sample. Finally, useful nucleic acids of the invention include recombinant expression plasmids for producing the nucleic acids of the invention. One especially useful type of such a plasmid is a plasmid used for *human* gene therapy. Useful plasmids of the invention for *human* gene therapy com[redacted] variety of types, including not only those that encode antisense oligonucleotides or ribozymes but also t[redacted]t drive expression of the RNA component of *human* telomerase* or a deleted or otherwise alter[redacted] (mutated) version of the RNA component of *human* (or other species with RNA component sequences substantially homologous to the *human* RNA component) *telomerase* or the gene for the same.
SUMM In a second aspect, the invention provides methods for treating a condition *associated* with the telomerase* activity within a cell or group of cells by contacting the cell(s) with a therapeutically effective amount of an agent that alters *telomerase* activity in that cell. Such agents include the *telomerase* RNA component-encoding nucleic acids, triple helix-forming oligonucleotides, antisense oligonucleotides, ribozymes, and plasmids for *human* gene therapy described above. In a related aspect, the invention provides pharmaceutical compositions comprising these therapeutic agents together with a. . .
SUMM In a fifth aspect, the invention provides methods for purifying the protein components of *human* telomerase* as well as the protein components of *telomerase* from a *mammalian* species with an RNA component substantially homologous to the RNA component of *human* *telomerase* . The present invention also provides methods for isolating and identifying nucleic acids encoding such protein components. In related aspects, the present invention provides purified *human* *telomerase* and purified *telomerase* of *mammalian* species with an RNA component substantially homologous to the RNA component of *human* *telomerase* , as well as purified nucleic acids that encode one or more components of such *telomerase* preparations. The present invention also provides pharmaceutical compositions comprising as an active ingredient the protein components of *telomerase* or a nucleic acid that encodes or *interacts* with a nucleic acid that encodes a protein component of *telomerase*.
SUMM The negative selection steps involved the preparation of biotinylated PCR product from cDNA prepared from a *human* cell line that does not have detectable *telomerase* activity. The biotinylated PCR product was denatured and then rehybridized in a solution comprising a much lower concentration of non-biotinylated PCR product (100 biotinylated product:1 non-biotinylated product) from cDNA prepared from a *human* cell line that does have *telomerase* activity. Given the possibility that the *telomerase* negative cell line might contain some low amount of the RNA component, the hybridization step was conducted to discriminate or. . . to a Co t selected to allow hybridization of the most abundantly expressed RNA, the unwanted material was removed by *binding* to streptavidinylated magnetic particles; the supernatant remaining after particle collection contained the desired cDNA for the RNA component of *human* *telomerase* . The process for PCR amplification of cDNA is described in Example 2, below.
SUMM . . . the invention is an antisense oligonucleotide that can be used in vivo or in vitro to inhibit the activity of *human* *telomerase* . Antisense oligonucleotides comprise a specific sequence of from about 10 to about 25 to 200 or more (i.e., large enough. . . delivery, to administer in vivo, if desired) nucleotides complementary to a specific sequence of nucleotides in the RNA component of *human* *telomerase* . The mechanism of action of such oligonucleotides can involve *binding* of the RNA component either to prevent assembly of the functional ribonucleoprotein *telomerase* or to prevent the RNA component from serving as a template for telomeric DNA synthesis.
SUMM Illustrative antisense oligonucleotides of the invention that serve to inhibit *telomerase* activity in vivo and/or in vitro include the oligonucleotides mentioned above in connection with the tests to determine whether clone pGRN7 comprised the cDNA for the RNA component of *human* *telomerase* . Three such oligonucleotides were synthesized as 2'-O-methyl RNA oligonucleotides, which *bind* more tightly to RNA than DNA oligonucleotides and are more resistant to hydrolysis than unmodified RNA oligonucleotides, and, as noted above, were used to demonstrate inhibition of *telomerase* activity in vitro. The sequence of each of these O-methyl RNA oligonucleotides is shown below.
SUMM . . . helix-forming oligonucleotides of the invention, *sense* oligonucleotides identical in sequence to at least a portion of the RNA component of *human* *telomerase* can also be used to inhibit *telomerase* activity. Oligonucleotides of the invention of this type are characterized in comprising either (1) less than the complete sequence of the RNA component needed to form a functional *telomerase* enzyme or (2) the complete sequence of the RNA component needed to form a functional *telomerase* enzyme as well as a substitution or insertion of one or more nucleotides that render the resulting RNA non-functional. In both cases, inhibition of *telomerase* activity is observed due to the *mutant* RNA component *binding* the protein components of *human* *telomerase* to form an inactive *telomerase* molecule. The mechanism of action of such oligonucleotides thus involves the assembly of a non-functional ribonucleoprotein *telomerase* or the prevention of assembly of a functional ribonucleoprotein *telomerase* . Sense oligonucleotides of the invention of this type typically comprise a specific sequence of from about 20, 50 200, 400, 500, or more nucleotides identical to a specific sequence of nucleotides in the RNA component of *human* *telomerase* .
SUMM Other oligonucleotides of the invention called *ribozymes* can also be used to inhibit *telomerase* activity. Unlike the antisense and other oligonucleotides described above, which *bind* to an RNA, a DNA, or a *telomerase* protein component, a ribozyme not only *binds* but also specifically cleaves and thereby potentially inactivates a target RNA, such as the RNA component of *human* *telomerase* . Such a ribozyme can comprise 5'- and 3'-terminal sequences complementary to the *telomerase* RNA. Depending on the site of cleavage, a ribozyme can render the *telomerase* enzyme inactive. See PCT patent publication No. 93/23572, supra. Those in the art upon review of the RNA sequence of the *human* *telomerase* RNA component will note that several useful ribozyme target sites are present and susceptible to cleavage by, for example, a. . .
SUMM Other therapeutic methods of the invention employ the *telomerase* RNA nucleic acid of the invention to stimulate *telomerase* activity and to extend replicative cell life span. These methods can be carried out by delivering to a cell a functional recombinant *telomerase* ribonucleoprotein of the invention to the cell. For instance, the ribonucleoprotein can be delivered to a cell in a liposome, or the gene for the RNA component of *human* *telomerase* (or a recombinant gene with different regulatory elements) can be used in a eukaryotic expression plasmid (with or without sequences coding for the expression of the protein components of *telomerase*) to activate *telomerase* activity in various normal *human* cells that otherwise lack detectable *telomerase* activity due to low levels of expression of the RNA component or a protein component of *telomerase* . If the *telomerase* RNA component is not sufficient to stimulate *telomerase* activity, then the RNA component can be transfected along with genes expressing the protein components of *telomerase* to stimulate *telomerase* activity. Thus, the invention provides methods for treating a condition *associated* with the *telomerase* activity within a cell or group of cells by contacting the cell(s) with a therapeutically effective amount of an agent that alters *telomerase* activity in that cell.
SUMM In related aspects, the invention features pharmaceutical compositions including a therapeutically effective amount of a *telomerase* inhibitor or *telomerase* activator of the invention. Pharmaceutical compositions of *telomerase* inhibitors of the invention include a mutant RNA component of *human* *telomerase* , an antisense oligonucleotide or triple helix-forming oligonucleotide that *binds* the RNA component or the gene for the same of *human* *telomerase* , or a ribozyme able to cleave the RNA component of *human* *telomerase* , or combinations of the same or other pharmaceuticals in a pharmaceutically acceptable carrier or salt. Other pharmaceutical compositions of the invention comprise a *telomerase* activator preparation, such as purified *human* *telomerase* or mRNA for the protein components of *telomerase* and the RNA component of *telomerase* , and are used to treat senescence-related disease. The therapeutic agent can be provided in a formulation suitable for parenteral, nasal,. . .
SUMM In addition, probes or primers that *bind* specifically to the RNA component of *human* *telomerase* (or either strand of the gene for the same) can be used in diagnostic methods to detect the presence of *telomerase* nucleic acid in a sample. Primers and probes are oligonucleotides that are complementary, and so will *bind* , to a target nucleic acid. Although primers and probes can differ in sequence and length, the primary differentiating factor is. . . one of function: primers serve to initiate DNA synthesis, as in PCR

amplification, while probes are typically used only to *bind* to a target nucleic acid. Typical lengths for a primer or probe can range from 8 to 20 to 30. . .

SUMM The reagents of the present invention also allow the cloning and isolation of nucleic acids encoding the protein components of *human* as well as other *mammalian* *telomerase* enzymes, which have not previously been available. Access to such nucleic acids provide complementary benefits to those provided by the nucleic acids comprising nucleic acid sequences of the RNA component of *human* *telomerase* . For instance, and as noted above, the therapeutic benefits of the present invention can be enhanced, in some instances, by use of purified preparations of the protein components of *human* *telomerase* and by access to nucleic acids encoding the same. The nucleic acids of the invention that encode the RNA component of human* *telomerase* can be used to isolate the nucleic acid encoding the protein components of *human* *telomerase* , allowing access to such benefits. Thus, the invention provides methods for isolating and purifying the protein components of *human* *telomerase* , as well as for identifying and isolating nucleic acids encoding the protein components of *human* *telomerase* . In related aspects, the present invention provides purified *human* *telomerase* , purified nucleic acids that encode the protein components of *human* *telomerase* , recombinant expression plasmids for the protein components of *human* *telomerase* . The invention also provides pharmaceutical compositions comprising as an active ingredient either the protein components of *human* *telomerase* or a nucleic acid that either encodes those protein components or *interacts* with nucleic acids that encode those protein components, such as antisense oligonucleotides, triple helix-froming oligonucleotides, ribozymes, or recombinant DNA expression. . .

SUMM The cloned RNA component of *human* *telomerase* can be used to identify and clone nucleic acids encoding the protein components of the ribonucleoprotein *telomerase* enzyme. Several different methods can be employed to achieve identification and cloning of the protein components. For instance, one can. . . enzyme or partially denatured enzyme using as an affinity ligand either (1) nucleotide sequences complementary to the RNA component to *bind* to the RNA component of the intact enzyme; or (2) the RNA component to *bind* the protein components of a partially or fully denatured enzyme. The ligand can be affixed to a solid support or chemically modified (e.g., biotinyiated) for subsequent immobilization on the support. Exposure of cell extracts containing *human* *telomerase* , followed by washing and elution of the *telomerase* enzyme bound to the support, provides a highly purified preparation of the *telomerase* enzyme. The protein components can then be optionally purified further or directly analyzed by protein sequencing. The protein sequence determined. . . cloning the cDNA or identifying a clone in a genomic bank comprising nucleic acids that encode a protein component of *telomerase* .

SUMM *Telomerase* RNA *binding* or *telomerase* activity assays for detection of specific *binding* proteins and activity can be used to facilitate the purification of the *telomerase* enzyme and the identification of nucleic acids that encode the protein components of the enzyme. For example, nucleic acids comprising. . . RNA component sequences can be used as affinity reagents to isolate, identify, and purify peptides, proteins or other compounds that *bind* specifically to a sequence contained within the RNA component, such as the protein components of *human* *telomerase* . Several different formats are available, including gel shift, filter *binding* , footprinting, Northwestern (RNA probe of protein blot), and photocrosslinking, to detect such *binding* and isolate the components that *bind* specifically to the RNA component. These assays can be used to identify *binding* proteins, to track purification of *binding* proteins, to characterize the RNA *binding* sites, to determine the molecular size of *binding* proteins, to label proteins for preparative isolation, and for subsequent immunization of animals for antibody generation to obtain antibodies for. . .

CLM What is claimed is:

34. An oligonucleotide probe or primer 10 to 30 nucleotides in length that specifically *binds* onlv to *human* *telomerase* RNA component or to either strand of a gene encoding *human* *telomerase* RNA component, wherein the oligonucleotide does not hybridize to telomeric DNA.

37. The oligonucleotide of claim 34 that specifically *binds* to the RNA component of *human* *telomerase* .

39. The oligonucleotide of claim 34 that specifically *binds* to a contiguous sequence contained within a *human* genomic DNA sequence encoding *human* *telomerase* RNA component located in an .about.2.5 kb HindIII-SacI insert of plasmid pGRN33 (ATCC 75926).

L15 ANSWER 4 OF 23 USPATFULL

SUMM While the methods of the invention are broadly applicable to the detection of *telomerase* activity in any sample from any origin, the methods are especially useful and applicable to the detection of *telomerase* activity in samples of biological material obtained from humans. Such samples will contain cells or cellular materials and will typically be obtained from humans for the purpose of detecting cancer. *Telomerase* is not expressed by normal post-natal *human* somatic cells, although low levels of *telomerase* activity can be detected in certain stem cells and activated cells of the hematopoietic system, so the presence of *telomerase* activity in a sample of *human* somatic tissue or cells indicates that immortal cells, including certain types of cancer cells, are present in the tissue. While not all cancer cells express *telomerase* activity, *telomerase* expression is required for cells to become immortal. Consequently, the presence of cells with *telomerase* activity is *associated* with many forms of cancer and can also serve to indicate that a particularly invasive or metastatic form of cancer. . .

DETD The requirement for the *telomerase* substrate to lack telomeric repeat sequences arises out of the second reaction of the present method--the non- *telomerase* -mediated primer extension reaction. In this reaction, an oligonucleotide primer that hybridizes only to extended *telomerase* substrates is added to the reaction mixture under conditions such that, if extended *telomerase* substrates are present, the primer *binds* to the extended substrates and is then extended by enzymatic action. Because *telomerase* can extend the *telomerase* substrate only by the addition of telomeric repeats, the primer will necessarily comprise a sequence complementary to a telomeric repeat. If the *telomerase* substrate employed in the *telomerase* extension reaction comprised a telomeric repeat, then the primer employed in the primer extension reaction could hybridize to unextended *telomerase* substrate, with potentially negative consequences. The *telomerase* substrate can, however, comprise sequences highly related to a telomeric repeat sequence without compromising the validity of the results obtained. For instance, an especially preferred *human* *telomerase* substrate of the invention is oligonucleotide M2, also known as TS, which contains a sequence at its 3'-end that is identical to five of the six bases of the *human* telomeric repeat but otherwise contains no telomeric repeat sequences.

DETD . . . type of any origin and can be used to detect an immortal cell of any origin, provided the cell expresses *telomerase* activity. For *human* samples, the detection of immortal cells will typically be used to detect the presence of cancer cells of any of. . . cell, papillary, scirrhous, bronchiolar, bronchogenic, squamous cell, and transitional cell), histiocytic disorders, leukemia (e.g., B-cell, mixed-cell, null-cell, T-cell, T-cell chronic, HTLV-II- *associated* , lymphocytic acute, lymphocytic chronic, mast-cell, and myeloid), histiocytosis malignant, Hodgkin's disease, immunoproliferative small, non-Hodgkin's lymphoma, plasmacytoma, reticuloendotheliosis, melanoma, chondroblastoma, chondroma,. . .

DETD The limit of *telomerase* detection in 102 cells was confirmed by TRAP assays of serial dilutions of an extract from 106 293 cells. This. . . NO:10)] or CTR4 [(5'-CCCTAA-3')4 (SEQ ID NO:11)] instead of CX further increases sensitivity, although these primers are more likely to *interact* with the unextended TS primer. The limit of sensitivity was also analyzed by titration of the synthetic *telomerase* product TS+4 (which contains oligonucleotide TS followed by four telomeric repeats). Dilutions of TS+4 oligonucleotide were mixed with heat-treated (*telomerase* inactivated) 293 extract and analyzed in TRAP assays. In this analysis, the assay gave a clear positive signal from 106 molecules of TS+4. In addition, *telomerase* activity from *mouse* tissue (*telomerase* activity is present in somatic cells of mice) and cell extracts was detected by TRAP assay even though the *mouse* *telomerase* by conventional assay was shown to be mostly non-processive (i.e., adds only a single repeat; Prowse et al., 1993, Natl. Acad. Sci. USA 90:1493-1497), indicating that the TRAP assay is detecting very low levels of product with *mouse* *telomerase* activity that cannot be visualized by the conventional assay.

L15 ANSWER 5 OF 23 USPATFULL

SUMM *Telomerase* is a *complex* of protein components and an integral RNA component. The RNA component of the *human* enzyme contains a short region complementary to the *human* telomeric repeat sequence (Feng et al., 269 Science 1236, 1995). This complementary sequence allows the *telomerase* RNA to serve as a template for the catalytic extension of the 3' telomeric termini (Greider et al., 337 Nature. . .

L15 ANSWER 6 OF 23 USPATFULL

SUMM A second method for the treatment of cellular senescence involves the use of an agent to derepress *telomerase* in cells where the enzyme is normally repressed. *Telomerase* activity is not detectable in any normal *human* somatic cells other than certain hemapoietic stem cells in vitro, but is detectable in cells that have abnormally reactivated the enzyme during the transformation of a normal cell into an immortal tumor cell. *Telomerase* activity may therefore be appropriate only in germ line cells and some stem cell populations such as hematopoetic stem cells.. . . Since the loss of telomeric repeats leading to senescence in somatic cells is occuring due to the absence of adequate *telomerase* activity, agents that have the effect of activating *telomerase* would have the effect of adding arrays of telomeric repeats to telomeres, thereby imparting to mortal somatic cells increased replicative. . . senescent cells the ability to proliferate and appropriately exit the cell cycle (in the absence of growth factor stimulation with *associated* appropriate regulation of cell cycle-linked genes typically inappropriately expressed in senescence e.g., collagenase, urokinase, and other secreted proteases and protease inhibitors). Such factors to derepress *telomerase* may be administered transiently or chronically to increase telomere length, and then removed, thereby allowing the somatic cells to again. . .

DRWD As noted above, the present invention concerns diagnosis and therapy *associated* with measuring telomeric length and manipulating *telomerase* -dependent extension or *telomerase* -independent shortening. While the invention is directed to humans, it may be applied to other animals, particularly *mammals* , such as other primates, and domestic animals, such as equine, bovine, avian, ovine, porcine, feline, and canine. The invention may. . . case of therapy, for example, telomere shortening may be slowed or inhibited by providing DNA oligonucleotides, by reactivating or introducing *telomerase* activity, or their functional equivalent, or indefinite proliferation can be reduced by inhibiting *telomerase* . In the case of diagnostics, one may detect the length of telomeres as to a particular chromosome or group of chromosomes, or the average length of telomeres. Diagnosis may also be *associated* with determining the activity of *telomerase* , or the presense of the components of the enzyme either on a protein or RNA level, in cells, tissue, and. . .

DRWD The nucleic acid sequences may be introduced into the cells as described previously. Various echniques exist to allow for depots *associated* with tumors. Thus, the inhibiting agents or nucleic acids may be administered as drugs, since they will only be effective only in cells which include *telomerase* . Since for the most part, *human* somatic cells lack *telomerase* activity, they will be unaffected. Some care may be required to prevent entry of such drugs into germ cells or some stem cell populations, which may express *telomerase* activity.

DRWD *Telomerase* activity is useful as a marker of growth potential, particularly as to neoplastic cells, or progenitor cells, e.g., embryonic stem cells. *Human* *telomerase* activity may be determined by measuring the rate of elongation of an appropriate repetitive sequence (primer), having 2 or more, usually 3 or more, repeats of the telomere unit sequence, TTAGGG. The sequence is labeled with a specific *binding* pair member at a convenient site, e.g., the 5'-terminus, and the specific *binding* pair member allows for separation of extended sequences. By using one or more radioactive nucleoside triphosphates or other labeled nucleoside. . . incorporated radioactivity as cpm per unit weight of DNA as a function of unit of time, as a measure of *telomerase* activity. Any other detectable signal and label may also be used, e.g., fluorescein.

L15 ANSWER 7 OF 23 USPATFULL

SUMM While the methods of the invention are broadly applicable to the detection of *telomerase* activity in any sample from any origin, the methods are especially useful and applicable to the detection of *telomerase* activity in samples of biological material obtained from humans. Such samples will contain cells or cellular materials and will typically. . . obtained from humans for the purpose of detecting a diseased state or other medical condition of interest, such as, cancer. *Telomerase* is not expressed by most normal post-natal *human* somatic cells, although low levels of *telomerase* activity can be detected in certain stem cells, activated cells of the hematopoietic system, and fetal tissues, so the presence of *telomerase* activity in a sample of *human* somatic tissue or cells indicates that cells of extended proliferative capacity, such as immortal cells, fetal cells, or hematopoietic cells, are present in the tissue. While not all cancer cells express *telomerase* activity, *telomerase* expression is required for cells to become immortal. Consequently, the presence of cells with *telomerase* activity is *associated* with many forms of cancer and can also serve to indicate that a particularly invasive or metastatic form of cancer. . .

DETD There is a requirement for the *telomerase* substrate to lack telomeric repeat sequences in some instances, in particular where the replication step of the present method involves the hybridization of a primer or probe to extended *telomerase* substrates. For example, in some embodiments, the non- *telomerase* -mediated primer extension reaction involves hybridization of an oligonucleotide primer that hybridizes only to extended *telomerase* substrates. This addition is made under conditions such that, if extended telomerase* substrates are present, the primer *binds* to the extended substrates and is then extended by enzymatic action. Because *telomerase* can extend the *telomerase* substrate only by the addition of telomeric repeats, the primer will necessarily comprise a sequence complementary to a telomeric repeat. If the *telomerase* substrate employed in the *telomerase* extension reaction comprised a complete telomeric repeat, then the primer employed in the primer extension reaction could hybridize readily to unextended *telomerase* substrate, with potentially negative consequences. The *telomerase* substrate can, however, comprise sequences highly related to a telomeric repeat sequence without compromising the validity of the results obtained. For instance, an especially preferred *human* *telomerase* substrate of the invention is oligonucleotide M2, also known as TS, which contains a sequence at its 3'-end that is identical to five of the six bases of the *human* telomeric repeat but otherwise contains no complete telomeric repeat sequences. There is no requirement that the *telomerase* substrate be free of telomeric repeat sequences where the replication or detection method is not compromised by the presence of. . .

DETD . . . type of any origin and can be used to detect an immortal cell of any origin, provided the cell expresses *telomerase* activity. For *human* samples, the detection of immortal cells will typically be used to detect the presence of cancer cells of any of. . . papillary, scirrhous, bronchiolar, bronchogenic, squamous cell, and transitional cell carcinomas), histiocytic disorders, leukemia (e.g., B-cell, mixed-cell, null-cell, T-cell, T-cell chronic, HTLV-II- *associated* , lymphocytic acute, lymphocytic chronic, mast-cell, and myeloid leukemias), histiocytosis malignant, Hodgkin's disease, immunoproliferative small, non-Hodgkin's lymphoma, plasmacytoma, reticuloendotheliosis, melanoma, chondroblastoma,. . .

DETD The limit of *telomerase* detection in 102 cells was confirmed by TRAP assays of serial dilutions of an extract from 106 293 cells. This. . . an absolute limit of the sensitivity of the current method. Example 15 is an illustrative example describing the detection of *telomerase* activity in a single cell. Those of skill in the art will recognize that other means of increasing the sensitivity. . . NO:17)] or CTR4 [(5'-CCCTAA-3')4 (SEQ ID NO:18)] instead of CX further increases sensitivity, although these primers are more likely to *interact* with the unextended TS primer. The limit of sensitivity was also analyzed by titration of the synthetic *telomerase* product TS+4 (which contains oligonucleotide TS followed by four telomeric repeats). Dilutions of TS+4 oligonucleotide were mixed with heat-treated (*telomerase* inactivated) 293 extract and analyzed in TRAP

assays. In this analysis, the assay gave a clear positive signal from 106 mole[illegible]of TS+4. In addition, *telomerase* activity from *mouse* tissue (*telomerase* activity is presen[illegible]atic cells of mice) and cell extracts was detected by TRAP assay even though the *mouse* *telomerase* [illegible] conventional assay was shown to be mostly non-processive (i.e., adds only a single repeat; Prowse et al., 1993, Proc. Natl. Acad. Sci. USA 90:1493-1497), indicating that the TRAP assay is detecting very low levels of processive *mouse* *telomerase* activity that cannot be visualized by the conventional assay or *mouse* *telomerase* is more processive under TRAP conditions.

L15 ANSWER 8 OF 23 USPATFULL

DETD . . . miltefosine; mirimostim; mismatched double stranded RNA; mitoguazone; mitolactol; mitomycin analogues; mitonafide; mitotoxin fibroblast growth factor-saporin; mitoxantrone; mofarotene; molgramostim; monoclonal antibody, *human* chorionic gonadotrophin; monophosphoryl lipid A +myobacterium cell wall sk; mopidamol; multiple drug resistance genie inhibitor; multiple tumor suppressor 1-based therapy;. . . perfosfamide; perillyl alcohol; phenazinomycin; phenylacetate; phosphatase inhibitors; picibanil; pilocarpine hydrochloride; pirarubicin; piritrexim; placetin A; placetin B; plasminogen activator inhibitor; platinum *complex* ; platinum compounds; platinum-triamine *complex* ; porfimer sodium; porfiromycin; propyl bis-acridone; prostaglandin J2; proteasome inhibitors; protein A-based immune modulator; protein kinase C inhibitor; protein kinase C. . . sargramostim; Sdi 1 mimetics; semustine; senescence derived inhibitor 1; sense oligonucleotides; signal transduction inhibitors; signal transduction modulators; single chain antigen *binding* protein; sizofiran; sobuzoxane; sodium borocaptate; sodium phenylacetate; solverol; somatomedin *binding* protein; sonermin; sparfosic acid; spicamycin D; spiromustine; splenopentin; spongistatin 1; squalamine; stem cell inhibitor; stem-cell division inhibitors; stipiamide; stromelysin inhibitors;. . . sulfmosine; superactive vasoactive intestinal peptide antagonist; suradista; suramin; swainsonine; synthetic glycosaminoglycans; tallimustine; tamoxifen methiodide; tauromustine; tazarotene; tecogalan sodium; tegafur; tellurapyrylium; *telomerase* inhibitors; temoporfin; temozolomide; teniposide; tetrachlorodecaoxide; tetrazomine; thaliblastine; thalidomide; thiocoraline; thrombopoietin; thrombopoietin mimetic; thymalfasin; thymopoietin receptor agonist; thymotrinan; thyroid stimulating hormone;. . .

L15 ANSWER 9 OF 23 USPATFULL

SUMM . . . first aspect, the present invention provides the RNA component of, as well as the gene for the NA component of, *human* *telomerase* in substantially pure form, as well as nucleic acids comprising all or at least a useful portion of the nucleotide sequence of the RNA component of *human* *telomerase* . The present invention also provides RNA component nucleic acids from other species, which nucleic acids share substantial homology with the RNA component of *human* *telomerase* , including but not limited to, the RNA components of *mammals* , such as primates. Other useful nucleic acids of the invention include nucleic acids with sequences complementary to the RNA component; nucleic acids with sequences related to but distinct from nucleotide sequences of the RNA component and which *interact* with the RNA component or the gene for the RNA component or the protein components of *human* *telomerase* in a useful way; and nucleic acids that do not share significant sequence homology or complementarity to the RNA component. . .

SUMM . . . or oligonucleotide mimetic (e.g., antisense PNA) that can be used in vivo or in vitro to inhibit the activity of *human* *telomerase* . Such oligonucleotides can block *telomerase* activity in a number of ways, including by preventing transcription of the *telomerase* gene (for instance, by triple helix formation) or by *binding* to the RNA component of *telomerase* in a manner that prevents a functional ribonucleoprotein *telomerase* from assembling or prevents the RNA component, once assembled into the *telomerase* enzyme *complex* , from serving as a template for telomeric DNA synthesis. Typically, and depending on mode of action, these oligonucleotides of the. . . or more nucleotides that is either identical or complementary to a specific sequence of nucleotides in the RNA component of *telomerase* or the gene for the RNA component of *telomerase* .

SUMM Another type of useful nucleic acid of the invention is a ribozyme able to cleave specifically the RNA component of *human* *telomerase* , rendering the enzyme inactive. Yet another type of useful nucleic acid of the invention is a probe or primer that *binds* specifically to the RNA component of *human* *telomerase* and so can be used, e.g., to detect the presence of *telomerase* in a sample. Finally, useful nucleic acids of the invention include recombinant expression plasmids for producing the nucleic acids of the invention. One especially useful type of such a plasmid is a plasmid used for *human* gene therapy. Useful plasmids of the invention for *human* gene therapy come in a variety of types, including not only those that encode antisense oligonucleotides or ribozymes but also those that drive expression of the RNA component of *human* *telomerase* or a deleted or otherwise altered (mutated) version of the RNA component of *human* (or other species with RNA component sequences substantially homologous to the *human* RNA component) *telomerase* or the gene for the same.

SUMM In a second aspect, the invention provides methods for treating a condition *associated* with the *telomerase* activity within a cell or group of cells by contacting the cell(s) with a therapeutically effective amount of an agent that alters *telomerase* activity in that cell. Such agents include the *telomerase* RNA component-encoding nucleic acids, triple helix-froming oligonucleotides, antisense oligonucleotides, ribozymes, and plasmids for *human* gene therapy described above. In a related aspect, the invention provides pharmaceutical compositions comprising these therapeutic agents together with a. . .

SUMM In a fifth aspect, the invention provides methods for purifying the protein components of *human* *telomerase* as well as the protein components of *telomerase* from a *mammalian* species with an RNA component substantially homologous to the RNA component of *human* *telomerase* . The present invention also provides methods for isolating and identifying nucleic acids encoding such protein components. In related aspects, the present invention provides purified *human* *telomerase* and purified *telomerase* of *mammalian* species with an RNA component substantially homologous to the RNA component of *human* *telomerase* , as well as purified nucleic acids that encode one or more components of such *telomerase* preparations. The present invention also provides pharmaceutical compositions comprising as an active ingredient the protein components of *telomerase* or a nucleic acid that encodes or *interacts* with a nucleic acid that encodes a protein component of *telomerase* .

DETD The negative selection steps involved the preparation of biotinylated PCR product from CDNA prepared from a *human* cell line that does not have detectable *telomerase* activity. The biotinylated PCR product was denatured and then rehybridized in a solution comprising a much lower concentration of non-biotinylated PCR product (100 biotinylated product:1 non-biotinylated product) from cDNA prepared from a *human* cell line that does have *telomerase* activity, Given the possibility that the *telomerase* negative cell line eight contain some low amount of the RNA component, the hybridization step was conducted to discriminate or. . . to a Co t selected to allow hybridization of the most abundantly expressed RNA, the unwanted material was removed by *binding* to streptavidinylated magnetic particles; the supernatant remaining after particle collection contained the desired cDNA for the RNA component of *human* *telomerase* . The process for PCR amplification of CDNA is described in Example 2, below.

DETD . . . the invention is an antisense oligonucleotide that can be used in vivo or in vitro to inhibit the activity of *human* *telomerase* . Antisense oligonucleotides comprise a specific sequence of from about 10 to about 25 to 200 or more (i.e., large enough. . . delivery, to administer in vivo, if desired) nucleotides complementary to a specific sequence of nucleotides in the RNA component of *human* *telomerase* . The mechanism of action of such oligonucleotides can involve *binding* of the RNA component either to prevent assembly of the functional ribonucleoprotein *telomerase* or to prevent the RNA component from serving as a template for telomeric DNA synthesis.

DETD Illustrative antisense oligonucleotides of the invention that serve to inhibit *telomerase* activity in vivo and/or in vitro include the oligonucleotides mentioned above in connection with the tests to determine whether clone pGRN7 comprised the cDNA for the RNA component of *human* *telomerase* . Three such oligonucleotides were synthesized as 2'-O[illegible]hyl RNA oligonucleotides, which *bind* more tightly to RNA than DNA oligonucleotides and are more res[illegible] hydrolysis than unmodified RNA oligonucleotides, and, as noted above, were used to demonstrate inhibition of *telomerase* activity in vitro. The sequence of each of these O-methyl RNA oligonucleotides is shown below.

DETD Additional embodiments directed to modulation of *telomerase* activity include methods that employ specific antisense polynucleotides complementary to all or part of the *human* *telomerase* RNA component (hTR) sequences, such as antisense polynucleotides to the *human* hTR gene or its transcribed RNA, including truncated forms which may be *associated* with *telomerase* holoenzyme. Such complementary antisense polynucleotides may include nucleotide substitutions, additions, deletions, or transpositions, so long as specific *binding* to the relevant target sequence corresponding to hTR or its gene is retained as a functional property of the polynucleotide.. . . by reference). The antisense polynucleotides therefore inhibit production of functional hTR. Since hTR expression (transcription rate and/or RNA stability) is *associated* with activation and enzymatic activity of *telomerase* holoenzyme, antisense polynucleotides that prevent transcription of RNA corresponding to hTR and/or the *interaction* of hTR to the protein component of *human* *telomerase* and/or the *interaction* of hTR to telomeric sequences may inhibit *telomerase* activity and/or reverse a phenotype, such as immortalization or neoplastic transformation, of cells expressing *telomerase* activity in the absence of antisense polynucelotides. Compositions containing a therapeutically effective dosage of hTR antisense polynucleotides may be administered for treatment of diseases which require *telomerase* activity for cellular pathogenesis (e.g., neoplasia) or to inhibit gamete production or maintenence (i.e., as a contraceptive), if desired. Antisense. . . consecutive nucleotides which are substantially complementary to a naturally-occurring hTR polynucleotide sequence, and typically which are perfectly complementary to a *human* hTR sequence, often being complementary to the sequence of hTR which is complemetary to the telomere repeat sequence, or complementary to a portion of the hTR which contacts the *telomerase* polypeptide subunit.

DETD . . . helix-forming oligonucleotides of the invention, *sense* oligonucleotides identical in sequence to at least a portion of the RNA component of *human* *telomerase* can also be used to inhibit *telomerase* activity. Oligonucleotides of the invention of this type are characterized in comprising either (1) less than the complete sequence of the RNA component needed to form a functional *telomerase* enzyme or (2) the complete sequence of the RNA component needed to form a functional *telomerase* enzyme as well as a substitution or insertion of one or more nucleotides that render the resulting RNA non-functional. In both cases, inhibition of *telomerase* activity is observed due to the *mutant* RNA component *binding* the protein components of *human* *telomerase* to form an inactive *telomerase* molecule. The mechanism of action of such oligonucleotides thus involves the assembly of a non-functional ribonucleoprotein *telomerase* or the prevention of assembly of a functional ribonucleoprotein *telomerase* . Sense oligonucleotides of the invention of this type typically comprise a specific sequence of from about 20, 50 200, 400, 500, or more nucleotides identical to a specific sequence of nucleotides in the RNA component of *human* *telomerase* .

DETD Other oligonucleotides of the invention called *ribozymes* can also be used to inhibit *telomerase* activity. Unlike the antisense and other oligonucleotides described above, which *bind* to an RNA, a DNA, or a *telomerase* protein component, a ribozyme not only *binds* but also specifically cleaves and thereby potentially inactivates a target RNA, such as the RNA component of *human* *telomerase* . Such a ribozyme can comprise 5'- and 3'-terminal sequences complementary to the *telomerase* RNA. Depending on the site of cleavage, a ribozyme can render the *telomerase* enzyme inactive. See PCT patent publication No. 93/23572, supra. Those in the art upon review of the RNA sequence of the *human* *telomerase* RNA component will note that several useful ribozyme target sites are present and susceptible to cleavage by, for example, a. . .

DETD Other therapeutic methods of the invention employ the *telomerase* RNA nucleic acid of the invention to stimulate *telomerase* activity and to extend replicative cell life span. These methods can be carried out by delivering to a cell a functional recombinant *telomerase* ribonucleoprotein of the invention to the cell. For instance, the ribonucleoprotein can be delivered to a cell in a liposome, or the gene for the RNA component of *human* *telomerase* (or a recombinant gene with different regulatory elements) can be used in a eukaryotic expression plasmid (with or without sequences coding for the expression of the protein components of *telomerase*) to activate *telomerase* activity in various normal *human* cells that otherwise lack detectable *telomerase* activity due to low levels of expression of the RNA component or a protein component of *telomerase* . If the *telomerase* RNA component is not sufficient to stimulate *telomerase* activity, then the RNA component can be transfected along with genes expressing the protein components of *telomerase* to stimulate *telomerase* activity. Thus, the invention provides methods for treating a condition *associated* with the *telomerase* activity within a cell or group of cells by contacting the cell(s) with a therapeutically effective amount of an agent that alters *telomerase* activity in that cell.

DETD In related aspects, the invention features pharmaceutical compositions including a therapeutically effective amount of a *telomerase* inhibitor or *telomerase* activator of the invention. Pharmaceutical compositions of *telomerase* inhibitors of the invention include a mutant RNA component of *human* *telomerase* , an antisense oligonucleotide or triple helix-forming oligonucleotide that *binds* the RNA component or the gene for the same of *human* *telomerase* , or a ribozyme able to cleave the RNA component of *human* *telomerase* , or combinations of the same or other pharmaceuticals in a pharmaceutically acceptable carrier or salt. Other pharmaceutical compositions of the invention comprise a *telomerase* activator preparation, such as purified *human* *telomerase* or RRNA for the protein components of *telomerase* and the RNA component of *telomerase* , and are used to treat senescence-related disease. In an aspect, a mutated sense hTR is administered to a cell population; said mutated sense hTR comprises at least one base mismatch with respect to the *human* *telomerase* repeat sequence, but is capable of exhibiting *telomerase* activity in conjunction with *human* *telomerase* polypeptide component,

producing misincorporation at selected nucleotide positions in the *human* *telomerase* repeat, thereby generating telomeres which rely on the continued presence of the mutated sense hTR for substantial replication. A therapeutic. . .

DETD In addition, probes or primers that *bind* specifically to the RNA component of *human* *telomerase* (or either strand of the gene for the same) can be used in diagnostic methods to detect the presence of *telomerase* nucleic acid in a sample. Primers and probes are oligonucleotides that are complementary, and so will *bind* , to a target nucleic acid. Although primers and probes can differ in sequence and length, the primary differentiating factor is. . . one of function: primers serve to initiate DNA synthesis, as in PCR amplification, while probes are typically used only to *bind* to a target nucleic acid. Typical lengths for a primer or probe can range from 8 to 20 to 30. . .

DETD The reagents of the present invention also allow the cloning and isolation of nucleic acids encoding the protein components of *human* as well as other *mammalian* *telomerase* enzymes, which have not previously been available. Access to such nucleic acids provide complementary benefits to those provided by the nucleic acids comprising nucleic acid sequences of the RNA component of *human* *telomerase* . For instance, and as noted above, the therapeutic benefits of the present invention can be enhanced, in some instances, by use of purified preparations of the protein components of *human* *telomerase* and by access to nucleic acids encoding the same. The nucleic acids of the invention that encode the RNA component of *human* *telomerase* can be used to isolate the nucleic acid encoding the protein components of *human* *telomerase* , allowing access to such benefits. Thus, the invention provides methods for isolating and purifying the protein components of *human* *telomerase* , as well as for identifying and isolating nucleic acids encoding the protein components of *human* *telomerase* . In related aspects, the present invention provides purified *human* *telomerase* , purified nucleic acids that encode the protein components of *human* *telomerase* , recombinant expression plasmids for the protein components of *human* *telomerase* . The invention also provides pharmaceutical compositions comprising as an active ingredient either the protein

components of *human* *telomerase* or a nucleic acid that either encodes [redacted]protein components or *interacts* with nucleic acids that encode those protein components, such [redacted]ense oligonucleotides, triple helix-froming oligonucleotides, ribozymes, or recombinant DNA expression. . .
DETD The cloned RNA component of *human* *telomerase* can be used to identify and clone nucleic acids encoding the protein components of the ribonucleoprotein *telomerase* enzyme. Several different methods can be employed to achieve identification and cloning of the protein components. For instance, one can. . . enzyme or partially denatured enzyme using as an affinity ligand either (1) nucleotide sequences complementary to the RNA component to *bind* to the RNA component of the intact enzyme; or (2) the RNA component to *bind* the protein components of a partially or fully denatured enzyme. The ligand can be affixed to a solid support or chemically modified (e.g., biotinylated) for subsequent immobilization on the support. Exposure of cell extracts containing *human* *telomerase* , followed by washing and elution of the *telomerase* enzyme bound to the support, provides a highly purified preparation of the *telomerase* enzyme. The protein components can then be optionally purified further or directly analyzed by protein sequencing. The protein sequence determined. . . cloning the cDNA or identifying a clone in a genomic bank comprising nucleic acids that encode a protein component of *telomerase* .
DETD *Telomerase* RNA *binding* or *telomerase* activity assays for detection of specific *binding* proteins and activity can be used to facilitate the purification of the *telomerase* enzyme and the identification of nucleic acids that encode the protein components of the enzyme. For example, nucleic acids comprising. . . RNA component sequences can be used as affinity reagents to isolate, identify, and purify peptides, proteins or other compounds that *bind* specifically to a sequence contained within the RNA component, such as the protein components of *human* *telomerase* . Several different formats are available, including gel shift, filter *binding* , footprinting, Northwestern (RNA probe of protein blot), and photocrosslinking, to detect such *binding* and isolate the components that *bind* specifically to the RNA component. These assays can be used to identify *binding* proteins, to track purification of *binding* proteins, to characterize the RNA *binding* sites, to determine the molecular size of *binding* proteins, to label proteins for preparative isolation, and for subsequent immunization of animals for antibody generation to obtain antibodies for. . .

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AB The invention provides methods and compositions rel a *human* *telomerase* and related nucleic acids, including four distinct *human* *telomerase* subunit proteins called p140, p105, p48 and p43 having *human* *telomerase* -specific activity. The proteins may be produced recombinantly from transformed host cells from the disclosed *telomerase* encoding nucleic acids or purified from *human* cells. Also included are *human* *telomerase* RNA components, as well as specific, functional derivatives thereof. The invention provides isolated *telomerase* hybridization probes and primers capable of specifically hybridizing with the disclosed *telomerase* gene, *telomerase* -specific *binding* agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the. . .
SUMM The invention provides isolated *human* *telomerase* proteins including *human* *telomerase* proteins p140, p105, p48 and p43, having molecular weights of about 149 kD, about 105 kD, about 48 kD and. . . kD, respectively, as determined by polyacrylamide gel electrophoresis under denaturing conditions (Matsudaira and Burgess (1978) Anal Biochem 87, 386-396), and *telomerase* protein domains thereof. The *telomerase* proteins comprise assay-discernable functional domains including RNA recognition motifs and subunit *binding* domains and may be provided as fusion products, e.g with non- *telomerase* polypeptides. The *human* *telomerase* proteins of the invention, including the subject protein domains, all have *telomerase* -specific activity or function.
SUMM *Telomerase* -specific activity or function may be determined by convenient in vitro, cell-based, or in vivo assays: e.g. in vitro *binding* assays, cell culture assays, in animals (e.g. immune response, gene therapy, transgenics, etc.), etc. *Binding* assays encompass any assay where the molecular *interaction* of a *telomerase* protein with a *binding* target is evaluated. The *binding* target may be a natural intracellular *binding* target such as a *telomerase* subunit (e.g. another protein subunit or RNA subunit), a substrate, agonist, antagonist, chaperone, or other regulator that directly modulates *telomerase* activity or its localization; or non-natural *binding* target such a specific immune protein such as an antibody, or a *telomerase* specific agent such as those identified in assays described below. Generally, *telomerase* -*binding* specificity is assayed by telomere polymerase activity (see, e.g. Collins et al. 1995, Cell 81, 677-686), by *binding* equilibrium constants (usually at least about 107 M-1, preferably at least about 108 M-1, more preferably at least about 109 M-1), by the ability of the subject protein to function as negative mutants in *telomerase* -expressing cells, to elicit *telomerase* specific antibody in a heterologous host (e.g a rodent or rabbit), etc. In any event, the *telomerase* *binding* specificity of the subject *telomerase* proteins necessarily distinguishes ciliate *telomerase* , preferably distinguishes non- *mammalian* telomerases and more preferably distinguishes non- *human* telomerases. Exemplary *telomerase* proteins which are shown to have *telomerase* *binding* specificity include the *telomerase* RNA (e.g. SEQ ID NO:6) *binding* domains (e.g. RRM 1-4: SEQ ID NO:1, about residues 5-81, residues 115-192, residues 336-420, and residues 487-578, respectively), *telomerase* primer *binding* domains, nucleotide triphosphate *binding* domains and *binding* domains of regulators of *telomerase* such as nuclear localization proteins, etc. As used herein, a protein domain comprises at least 12, preferably at least about. . .
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SUMM The amino acid sequences of the disclosed *telomerase* proteins are used to backtranslate telomerase* protein-encoding nucleic acids optimized for selected expression systems (Holler et al. (1993) Gene 136, 323-328; Martin et al. (1995) Gene 154, 150-166) or used to generate degenerate oligonucleotide primers and probes for use in the isolation of natural *telomerase* encoding nucleic acid sequences ("GCG" software, Genetics Computer Group, Inc, Madison Wis.). As examples, SEQ ID NO:2 discloses an ambiguity-maximized p105 coding sequence encompassing all possible nucleic acids encoding the full-length p105 protein. SEQ ID NO:3 discloses a natural *human* cDNA sequence encoding p105, SEQ ID NO:4 is a p105
coding sequence codon-optimized for E. coli, SEQ ID NO:5 is a p105 coding sequence codon optimized for *mammalian* cell expression. *Telomerase* encoding nucleic acids may be part of *human* *telomerase* -expression vectors and may be incorporated into recombinant host cells, e.g. for expression and screening, transgenic animals, e.g. for functional st[redacted]such as the efficacy of candidate drugs for disease *associated* with *human* *telomerase* -mediated [redacted]ransduction, etc. Expression systems are selected and/or tailored to effect *human* *telomerase* protein structural and functional variants through alternative post-translational processing.
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SUMM. . . are of synthetic/non-natural sequences and/or are isolated, i.e. unaccompanied by at least some of the material with which it is *associated* in its natural state, preferably constituting at least about 0.5%, preferably at least about 5% by weight of total nucleic. . . applications including use as translatable transcripts, hybridization probes, PCR primers, diagnostic nucleic acids, etc.; use in detecting the presence of *human* *telomerase* genes and gene transcripts and in detecting or amplifying nucleic acids encoding additional *human* *telomerase* homologs and structural analogs.
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SUMM The resultant mixture is incubated under conditions whereby, but for the presence of the candidate pharmacological agent, the *human* *telomerase* specifically *binds* the cellular *binding* target, portion or analog with a reference *binding* affinity. The mixture components can be added in any order that provides for the requisite *bindings* and incubations may be performed at any temperature which facilitates optimal *binding* . Incubation periods are likewise selected for optimal *binding* but also minimized to facilitate rapid, high-throughput screening.
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SUMM A difference in the *binding* affinity of the *human* *telomerase* protein to the target in the absence of the agent as compared with the *binding* affinity in the presence of the agent indicates that the agent modulates the *binding* of the *human* *telomerase* protein to the *human* *telomerase* *binding* target. Analogously, in the cell-based transcription assay also described below, a difference in the *human* *telomerase* transcriptional induction in the presence and absence of an agent indicates the agent modulates *human* *telomerase* -induced transcription. A difference, as used herein, is statistically significant and preferably represents at least a 50%, more preferably at least. . .
DETD 2. Protocol for High Throughput *Human* *Telomerase* Subunit-RNA *Complex* Formation Assay

L15 ANSWER 11 OF 23 USPATFULL
TI *Human* *telomerase* RNA *interacting* protein gene
AB The invention provides methods and compositions relating to a *human* *telomerase* and related nucleic acids, including four distinct *human* *telomerase* subunit proteins called p140, p105, p48 and p43 having *human* telomerae-specific activity. The proteins may be produced recombinantly from transformed host cells from the disclosed *telomerase* encoding nucleic acids or purified from *human* cells. Also included are *human* *telomerase* RNA components, as well as specific, functional derivative thereof. The invention provides isolated *telomerase* hybridization probes and primers capable of specifically hybridizing with the disclosed *telomerase* gene, *telomerase* -specific *binding* agents such as specific antibodies, and methods of making and using the subject compositions in diagnosis, therapy and in the. . .
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SUMM A difference in the *binding* affinity of the *human* *telomerase* protein to the target in the absence of the agent as compared with the *binding* affinity in the presence of the agent indicates that the agent modulates the *binding* of the *human* *telomerase* protein to the *human* *telomerase* *binding* target. Analogously, in the cell-based transcription assay also described below, a difference in the *human* *telomerase* transcriptional induction in the presence and absence of an agent indicates the agent modulates *human* *telomerase* -induced transcription. A difference, as used herein, is statistically significant and preferably represents at least a 50%, more preferably at least. . .

DETD 2. Protocol for high throughput *human* *telomerase* subunit-RNA *complex* formation assay.

L15 ANSWER 12 OF 23 USPATFULL

SUMM Hanish et al (Proc. Natl. Acad. Sci. USA 91:8861-8865) examined the requirements for the formation of *human* telomeres from TTAGGG seeds, and found that telomere formation was not correlated with the ability of *human* *telomerase* to elongate telomeric sequences in vitro, and did not appear to be a result of homologous recombination. Rather, the investigators reported that the sequence dependence of telomere formation matched the in vitro *binding* requirements for TRF.

DETD . . . repeats. This telomeric nucleotide sequence is specified by the 5' CUAACC 3' template within the RNA component of vertebrate telomerases. *Telomerase* -mediated addition of GGTTAG repeats to the 3' chromosome ends can balance the loss of terminal sequences that occurs during replication of linear DNAs. The telomeric repeat array is essential for the stability of *mammalian* chromosomes. Without this protective cap, chromosome termini might activate DNA damage checkpoints, or be attacked by DNA repair functions leading to chromosome end fusion and degradation. This protective function of vertebrate telomeres is thought to result from the *interaction* of the telomeric GGTTAG repeats with telomere specific proteins. In support of this notion, telomeres in yeast and hypotrichous ciliates. . . chromatin structure (Tommerup et al (1994) Mol. Cell Biol. 14:5777-5785), and the sequence requirements for formation of new telomeres in *human* cells are highly specific (Hanish et al (1994) Proc. Natl. Acad. Sci. USA 91:8861-8865), as would be expected if the GGTTAG repeats *interact* with a sequence specific DNA *binding* protein (de Lange (1995) Seminars in Cell Biology 7, in press).

L15 ANSWER 13 OF 23 USPATFULL

SUMM It has been known for some years that telomeres in *human* germline cells (e.g. sperm) are longer than those in somatic tissue such as blood. One proposed explanation for this is the absence of telomere repeat addition (i.e. absence of *telomerase* activity) in somatic cells. If so, incomplete end replication would be expected to result in the progressive loss of terminal. . . contribute to the chromosome aberrations typically seen in senescent cells. Senescence and the measurement of cellular time is an intriguingly *complex* subject and it will be interesting to see to what extent telomere shortening has a causal role. The large telomeres. . .

SUMM . . . special structure and behavior of telomeric DNA, suggest that telomere synthesis could be a target for selective drug action. Because *telomerase* activity seems to be essential for protozoans or yeast, but not apparently for *mammalian* somatic cells, I propose that *telomerase* should be explored as a target for drugs against eukaryotic pathogenic or parasitic microorganisms, such as parasitic protozoans or pathogenic yeasts. A drug that *binds* *telomerase* selectively, either through its reverse-transcriptase or DNA substrate-*binding* properties, should selectively act against prolonged maintenance of the dividing lower eukaryote, but not impair the *mammalian* host over the short term, because *telomerase* activity in its somatic cells may normally be low or absent. Obvious classes of drugs to investigate are those directed specifically against reverse transcriptases as opposed to other DNA or RNA polymerases, and drugs that would *bind* telomeric DNA itself. These could include drugs that selectively *bind* the G.G base-paired forms of the G-rich strand protrusions at the chromosome termini, or agents which stabilize an inappropriate G.G. . .

DETD As noted above, the present invention concerns diagnosis and therapy *associated* with measuring telomeric length and manipulating *telomerase* -dependent extension or *telomerase* -independent shortening. While the invention is directed to humans, it may be applied to other animals, particularly *mammals* , such as other primates, and domestic animals, such as equine, bovine, ovine, porcine, feline, and canine. The invention may be. . . may be slowed or inhibited by providing DNA oligonucleotides or their functional equivalent, or self-proliferation can be reduced by inhibiting *telomerase* . In this case of diagnostics, one may detect the length of telomeres as to a particular chromosome or group of chromosomes, or the average length of telomeres. iagnosis may also be *associated* with determining the activity of *telomerase* in cells, tissue, and the like.

DETD *Telomerase* activity is of interest as a marker of growth potential, particularly as to neoplastic cells, or progenitor cells, e.g., embryonic cells. *Human* *telomerase* activity may be determined by measuring the rate of elongation of an appropriate repetitive sequence (primer), having 2 or more, usually 3 or more, repeats of the telomere unit sequence, TTAGGG. The sequence is labeled with a specific *binding* pair member at a convenient site, e.g., the 5' -terminus, which specific *binding* pair member allows for separation of extended sequences. By using one or more radioactive nucleotide triphosphates or other labeled nucleotide. . . ncorporated radioactivity as cpm per unit weight of DNA as a function of unit of time, as a measure of *telomerase* activity. Any other detectable signal and label may also be used, e.g., fluorescein.

L15 ANSWER 14 OF 23 USPATFULL

SUMM As used herein, the term " *telomerase* component" refers to a biological component that is *associated* with a non-ciliate eukaryotic *telomerase* *complex* , such as a *mammalian* , drosophila or yeast *telomerase* component. Preferably, the *telomerase* components will be *associated* with a yeast *telomerase* *complex* . A " *telomerase* *complex* " in this sense is a ribonucleoprotein enzyme *complex* that functions to elongate the G-rich strand of eukaryotic, and preferably yeast, chromosomal termini by adding telomeric repeats. *Telomerase* components (or *telomerase* - *associated* components) therefore include both RNA and polypeptidyl components.

SUMM An important component of *telomerase* is the *telomerase* RNA template or template sequence. The term " *telomerase* RNA template", as used herein, refers to a non-ciliate eukaryotic, such as a *mammalian* , drosophila, or preferably, a yeast *telomerase* RNA component that includes a sequence that is complementary to the telomere repeat, i.e., that is complementary to the G-rich or GT-rich sequences of chromosomal termini. The *telomerase* RNA template is thus an isolated RNA component that has a C-rich or CA-rich sequence and that, by *interacting* with other *telomerase* components, functions to extend telomeric repeats. The *telomerase* RNA template may also be defined as the *telomerase* substrate for reverse transcription.

SUMM . . . to a DNA molecule that has been isolated free of total genomic DNA of a particular species, such as a *mammal* , drosophila or yeast species. Therefore, a DNA segment that comprises a sequence region that encodes a *telomerase* - *associated* component refers to a DNA segment that includes *telomerase* - *associated* component coding sequences or regions, yet is isolated away from, or purified free from, total genomic DNA of the species. . .

SUMM Similarly, a *telomerase* - *associated* gene is a DNA segment comprising an isolated or purified gene

that includes a sequence region that encodes a component *associated* wi[redacted]mmalian* , drosophila, or preferably, with a yeast *telomerase* . The term "an isolated gene *associa[redacted]th a non-ciliate eukaryotic *telomerase* ", as used herein, refers to a DNA segment including *telomerase* RNA or protein coding sequences or regions and, in certain aspects, regulatory sequences, isolated substantially away from other naturally occurring. . .

SUMM In further embodiments, the invention provides isolated DNA segments, genes and vectors incorporating DNA sequences that encode a non-ciliate eukaryotic *telomerase* - *associated* polypeptide, such as a *mammalian* , drosophila or yeast, *telomerase* - *associated* polypeptide, as exemplified by yeast polypeptides that includes within their amino acid sequence a contiguous amino acid sequence from SEQ. . .

SUMM . . . promoter. As used herein, a recombinant or heterologous promoter is intended to refer to a promoter that is not normally *associated* with a *telomerase* - *associated* gene in its natural environment. Such promoters may include yeast promoters normally *associated* with other genes, and/or promoters isolated from any other bacterial, viral, insect or *mammalian* cell.

SUMM The invention further concerns recombinant RNA segments that include non-ciliate *telomerase* RNA templates, such as *mammalian* , drosophila, or preferably, yeast *telomerase* RNA templates; and recombinant protein and polypeptide compositions, free from total cell components, that comprise one or more purified non-ciliate, or preferably, yeast *telomerase* - *associated* components. These are exemplified by polypeptides that include a contiguous amino acid sequence from SEQ ID NO:16, SEQ ID NO:18,. . .

SUMM These detection methods may be employed to detect *telomerase* - *associated* genes, whether RNA- or protein-encoding, in both clinical and laboratory samples, e.g., as may be used in *telomerase* purification, analysis, mutagenesis and the like. In cells or cellular extracts obtained from an animal or *human* patient, the detection of *telomerase* may have particular relevance, for example, in the diagnosis or detection of tumor cells within a sample suspected of containing such cells. This is supported by recent findings linking *telomerase* to oncogenesis and various late stage tumors and tumor cells (Harley et al., 1992; Counter et al., 1992, 1994a; Shay. . .

SUMM Further clinical samples that may be analyzed for the presence of *telomerase* - *associated* genes, as described above, include those suspected of containing a pathogen. As *telomerase* activity is only present in dividing cells, testing a sample of somatic cells of an animal or *human* for the presence of *telomerase* may indicate the presence of an invading unicellular organism within the sample. This may allow disease diagnosis alone, or in. . .

SUMM Diagnostic methods for identifying various conditions *associated* with infertility in animals and humans are also provided by the invention. For example, as *telomerase* activity is required in germ cells, including *human* sperm and ova, testing samples from animals and humans suspected of having a condition connected with reproductive failure would provide. . .

SUMM One suitable method for identifying a *human* *telomerase* - *associated* gene, is to apply the suppression of telomeric silencing protocol to a *human* nucleic acid library using a yeast cell system. Such methods generally comprise preparing a yeast cell containing a chromosome that. . . a telomere, where the telomere represses the expression of the marker; contacting the cell with a composition comprising a candidate *human* gene; and identifying a *human* gene that allows expression of the marker.

SUMM Further suitable methods for identifying *human* *telomerase* - *associated* genes are those based entirely upon *human* cells, which methods presuppose the lowest level of homology between the yeast and *human* cell systems. These methods comprise preparing a *human* cell that contains a chromosome having a genetic marker located proximal to a telomere, where the telomere represses the expression of the marker; contacting the cell with a composition comprising a candidate *human* gene; and identifying a *human* gene that allows expression of the marker.

SUMM . . . to the nucleic acid hybridization methods described hereinabove. Indeed, the "candidate substances" to be detected may be nucleic acids, including *human* nucleic acid segments, that are detected by *binding* to eukaryotic, and preferably, to yeast *telomerase* RNA or DNA components, and preferably to a defined small functional region of the template that suppress silencing, under the high or low hybridization conditions described above. However, other components that *bind* to *telomerase* may be identified by *binding* to the isolated RNA, DNA or polypeptide components of the present invention. These components may include proteins, polypeptides, peptides, antibodies,. . .

DETD . . . used as hosts. In principle, any such cell culture is workable, whether from vertebrate or invertebrate culture. In addition to *mammalian* cells, these include insect cell systems infected with recombinant virus expression vectors (e.g., baculovirus); and plant cell systems infected with. . . cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with recombinant plasmid expression vectors (e.g., Ti plasmid) containing the *telomerase* - *associated* coding sequences.

DETD . . . reference. Typically, this technique involves immunizing a suitable animal with a selected immunogen composition, e.g., a purified or partially purified *telomerase* - *associated* protein, polypeptide or peptide. The immunizing composition is administered in a manner effective to stimulate antibody producing cells. Rodents such. . . possible. The use of rats may provide certain advantages (Goding, 1986, pp. 60-61), but mice are preferred, with the BALB/c *mouse* being most preferred as this is most routinely used and generally gives a higher percentage of stable fusions.

L15 ANSWER 15 OF 23 USPATFULL

SUMM "It has been known for some years that telomeres in *human* germline cells (e.g. sperm) are longer than those in somatic tissue such as blood. One proposed explanation for this is the absence of telomere repeat addition (i.e. absence of *telomerase* activity) in somatic cells. If so, incomplete end replication would be expected to result in the progressive loss of terminal. . . contribute to the chromosome aberrations typically seen in senescent cells. Senescence and the measurement of cellular time is an intriguingly *complex* subject and it will be interesting to see to what extent telomere shortening has a causal role. The large telomeres. . .

SUMM . . . special structure and behavior of telomeric DNA, suggest that telomere synthesis could be a target for selective drug action. Because *telomerase* activity seems to be essential for protozoans or yeast, but not apparently for *mammalian* somatic cells, I propose that *telomerase* should be explored as a target for drugs against eukaryotic pathogenic or parasitic microorganisms, such as parasitic protozoans or pathogenic yeasts. A drug that *binds* *telomerase* selectively, either through its reverse-transcriptase or DNA substrate-*binding* properties, should selectively act against prolonged maintenance of the dividing lower eukaryote, but not impair the *mammalian* host over the short term, because *telomerase* activity in its somatic cells may normally be low or absent. Obvious classes of drugs to investigate are those directed specifically against reverse transcriptases as opposed to other DNA or RNA polymerases, and drugs that would *bind* telomeric DNA itself. These could include drugs that selectively *bind* the G.degree.G base-paired forms of the G-rich strand protrusions at the chromosome termini, or agents which stabilize an inappropriate G.degree.G. . .

SUMM A second method for the treatment of cellular senescence involves the use of an agent to derepress *telomerase* in cells where the enzyme is normally repressed. *Telomerase* activity is not detectable in any normal *human* somatic cells, but is detectable in cells that have abnormally reactivated the enzyme during the transformation of a normal cell into an immortal tumor cell. *Telomerase* activity may therefore be appropriate only in germ line cells and some stem cell populations (though there is currently no evidence of the latter in *human* tissues). Since the loss of telomeric repeats leading to senescence in somatic cells is occuring due to the absence of adequate *telomerase* activity, agents that have the effect of activating *telomerase* would have the effect of adding arrays of telomeric repeats to telomeres, thereby imparting to mortal somatic gells increased replicative. . . senescent cells the ability to proliferate and appropriately exit the cell cycle (in the absence of growth factor stimulation with *associated* appropriate regulation of cell cycle-linked genes typically inappropriately expressed in senescence e.g., collagenase, urokinase, and other secreted proteases and rotease inhibitors). Such factors to derepress *telomerase* may be administered transiently or chronically to increase telomere length, and then removed, thereby allowing the somatic cells to again. . .

DETD As noted above, the present inve[redacted]oncerns diagnosis and therapy *associated* with measuring telomeric length and manipulating *tel[redacted]e* -dependent extension or *telomerase* -independent shortening. While the invention is directed to humans, it may be applied to other animals, particularly *mammals* , such as other primates, and domestic animals, such as equine, bovine, avian, ovine, porcine, feline, and canine. The invention may. . . case of therapy, for example, telomere shortening may be slowed or inhibited by providing DNA oligonucleotides by reactivating or introducing *telomerase* activity, or their functional quivalent, or indefinite proliferation can be reduced by inhibiting *telomerase* . In the case of diagnostics, one may detect the length of telomeres as to a particular chromosome or group of chromosomes, or the average length of telomeres. Diagnosis may also be *associated* with determining the activity of *telomerase* , or the presense of the components of the enzyme either on a protein or RNA level, in cells, tissue, and. . .

DETD The nucleic acid sequences may be introduced into the cells as described previously. Various techniques exist to allow for depots *associated* with tumors. Thus, the inhibiting agents or nucleic acids may be administered as drugs, since they will only be effective only in cells which include *telomerase* . Since for the most part, *human* somatic cells lack *telomerase* activity they will be unaffected. Some care may be required to prevent entry of such drugs into germ cells, which way express *telomerase* activity.

DETD "Telomerase" activity is useful as a marker of growth potential, particularly as to neoplastic cells, or progenitor cells, e.g., embryonic stem cells. *Human* *telomerase* activity may be determined by measuring the rate of elongation of an appropriate repetitive sequence (primer), having 2 or more, usually 3 or more, repeats of the telomere unit sequence, TTAGGG. The sequence is labeled with a specific *binding* pair member at a convenient site, e.g., the 5'-terminus, and the specific *binding* pair member allows for separation of extended sequences. By using one or more radioactive nucleoside triphosphates or other labeled nucleoside. . . incorporated radioactivity as cpm per unit weight of DNA as a function of unit of time, as a measure of telomerase* activity. Any other detectable signal and label may also be used, e.g., fluorescein.

L15 ANSWER 16 OF 23 USPATFULL

DETD "Human" fetal adrenal gland, ovary, testis, and brain tissues were obtained from normal fetuses at 16 and 18 weeks of gestational. . . died of complications of surgery, and a 5-year old child who had died with CNS abnormalities and congenital heart disease *associated* with Pena-Shokier syndrome. Normal adult testis and other tissues were obtained from a 37-year old male who had died of acute heart failure. All tissues were stored at -80.degree. C. until used. Cell extracts and *telomerase* activity assays were carded out as described in Example 1.

L15 ANSWER 17 OF 23 USPATFULL

SUMM It has been known for some years that telomeres in *human* germline cells (e.g. sperm) are longer than those in somatic tissue such as blood. One proposed explanation for this is the absence of telomere repeat addition (i.e. absence of *telomerase* activity) in somatic cells. If so, incomplete end replication would be expected to result in the progressive loss of terminal. . . contribute to the chromosome aberrations typically seen in senescent cells. Senescence and the measurement of cellular time is an intriguingly *complex* subject and it will be interesting to see to what extent telomere shortening has a causal role. The large telomeres. . .

SUMM . . . special structure and behavior of telomeric DNA, suggest that telomere synthesis could be a target for selective drug action. Because *telomerase* activity seems to be essential for protozoans or yeast, but not apparently for *mammalian* somatic cells, I propose that *telomerase* should be explored as a target for drugs against eukaryotic pathogenic or parasitic microorganisms, such as parasitic protozoans or pathogenic yeasts. A drug that *binds* *telomerase* selectively, either through its reverse-transcriptase or DNA substrate-*binding* properties, should selectively act against prolonged maintenance of the dividing lower eukaryote, but not impair the *mammalian* host over the short term, because *telomerase* activity in its somatic cells may normally be low or absent. Obvious classes of drugs to investigate are those directed specifically against reverse transcriptases as opposed to other DNA or RNA polymerases, and drugs that would *bind* telomeric DNA itself. These could include drugs that selectively *bind* the G.multidot.G base-paired forms of the G-rich strand protrusions at the chromosome termini, or agents which stabilize an inappropriate G.multidot.G. . .

DETD As noted above, the present invention concerns diagnosis and therapy *associated* with measuring telomeric length and manipulating *telomerase* -dependent extension or *telomerase* -independent shortening. While the invention is directed to humans, it may be applied to other animals, particularly *mammals* , such as other primates, and domestic animals, such as equine, bovine, ovine, porcine, feline, and canine. The invention may be. . . may be slowed or inhibited by providing DNA oligonucleotides or their functional equivalent, or self-proliferation can be reduced by inhibiting *telomerase* . In this case of diagnostics, one may detect the length of telomeres as to a particular chromosome or group of chromosomes, or the average length of telomeres. Diagnosis may also be *associated* with determining the activity of *telomerase* in cells, tissue, and the like.

DETD "Telomerase" activity is of interest as a marker of growth potential, particularly as to neoplastic cells, or progenitor cells, e.g., embryonic cells. *Human* *telomerase* activity may be determined by measuring the rate of elongation of an appropriate repetitive sequence (primer), having 2 or more, usually 3 or more, repeats of the telomere unit sequence, TTAGGG. The sequence is labeled with a specific *binding* pair member at a convenient site, e.g., the 5'-terminus, which specific *binding* pair member allows for separation of extended sequences. By using one or more radioactive nucleotide triphosphates or other labeled nucleotide. . . incorporated radioactivity as cpm per unit weight of DNA as a function of unit of time, as a measure of *telomerase* activity. Any other detectable signal and label may also be used, e.g., fluorescein.

L15 ANSWER 18 OF 23 USPATFULL

SUMM "It has been known for some years that telomeres in *human* germline cells (e.g. sperm) are longer than those in somatic tissue such as blood. One proposed explanation for this is the absence of telomere repeat addition (i.e. absence of *telomerase* activity) in somatic cells. If so, incomplete end replication would be expected to result in the progressive loss of terminal. . . contribute to the chromosome aberrations typically seen in senescent cells. Senescence and the measurement of cellular time is an intriguingly *complex* subject and it will be interesting to see to what extent telomere shortening has a causal role. The large telomeres. . .

SUMM . . . special structure and behavior of telomeric DNA, suggest that telomere synthesis could be a target for selective drug action. Because *telomerase* activity seems to be essential for protozoans or yeast, but not apparently for *mammalian* somatic cells, I propose that *telomerase* should be explored as a target for drugs against eukaryotic pathogenic or parasitic microorganisms, such as parasitic protozoans or pathogenic yeasts. A drug that *binds* *telomerase* selectively, either through its reverse-transcriptase or DNA substrate-*binding* properties, should selectively act against prolonged maintenance of the dividing lower eukaryote, but not impair the *mammalian* host over the short term, because *telomerase* activity in its somatic cells may normally be low or absent. Obvious classes of drugs to investigate are those directed specifically against reverse transcriptases as opposed to other DNA or RNA polymerases, and drugs that would *bind* telomeric DNA itself. These could include drugs that selectively *bind* the G.degree.G base-paired forms of the G-rich strand protrusions at the chromosome termini, or agents which stabilize an inappropriate G.degree.G. . .

SUMM A second method for the treatment of cellular senescence involves the use of an agent to derepress *telomerase* in cells where the enzyme is normally repressed. *Telomerase* activity is not detectable in any normal *human* somatic cells other than certain hemapoietic stem cells in vitro, but is detectable in cells that have abnormally reactivated the enzyme during the transformation of a normal cell into an immortal tumor cell. *Telomerase* activity may therefore be appropriate only in germ line cells and some stem cell populations such as hematopoetic stem cells.. . . Since the loss of telomeric repeats leading to senescence in somatic cells is occuring due to the absence of adequate *telomerase* activity, agents that have the effect of activating *telomerase* would have the effect of adding arrays of telomeric repeats to telomeres, thereby imparting to mortal somatic cells increased replicative. . . senescent cells the ability to proliferate and appropriately exit

the cell cycle (in the absence of growth factor stimulation with *associated* [redacted]riate regulation of cell cycle-linked genes typically inappropriately expressed in senescence e.g., c[redacted]ase, urokinase, and other secreted proteases and protease inhibitors). Such factors to derepress *telomerase* may be administered transiently or chronically to increase telomere length, and then removed, thereby allowing the somatic cells to again. . .

DETD As noted above, the present invention concerns diagnosis and therapy *associated* with measuring telomeric length and manipulating *telomerase* -dependent extension or *telomerase* -independent shortening. While the invention is directed to humans, it may be applied to other animals, particularly *mammals* , such as other primates, and domestic animals, such as equine, bovine, avian, ovine, porcine, feline, and canine. The invention may. . . case of therapy, for example, telomere shortening may be slowed or inhibited by providing DNA oligonucleotides, by reactivating or introducing *telomerase* activity, or their functional equivalent, or indefinite proliferation can be reduced by inhibiting *telomerase* . In the case of diagnostics, one may detect the length of telomeres as to a particular chromosome or group of chromosomes, or the average length of telomeres. Diagnosis may also be *associated* with determining the activity of *telomerase* , or the presense of the components of the enzyme either on a protein or RNA level, in cells, tissue, and. . .

DETD The nucleic acid sequences may be introduced into the cells as described previously. Various techniques exist to allow for depots *associated* with tumors. Thus, the inhibiting agents or nucleic acids may be administered as drugs, since they will only be effective only in cells which include *telomerase* . Since for the most part, *human* somatic cells lack *telomerase* activity, they will be unaffected. Some care may be required to prevent entry of such drugs into germ cells or some stem cell populations, which may express *telomerase* activity.

DETD *Telomerase* activity is useful as a marker of growth potential, particularly as to neoplastic cells, or progenitor cells, e.g., embryonic stem cells. *Human* *telomerase* activity may be determined by measuring the rate of elongation of an appropriate repetitive sequence (primer), having 2 or more, usually 3 or more, repeats of the telomere unit sequence, TTAGGG. The sequence is labeled with a specific *binding* pair member at a convenient site, e.g., the 5'-terminus, and the specific *binding* pair member allows for separation of extended sequences. By using one or more radioactive nucleoside triphosphates or other labeled nucleoside. . . incorporated radioactivity as cpm per unit weight of DNA as a function of unit of time, as a measure of *telomerase* activity. Any other detectable signal and label may also be used, e.g., fluorescein.

L15 ANSWER 19 OF 23 USPATFULL

SUMM In contrast, the present invention employs a single-stranded synthetic oligonucleotide as a telomere motif decoy (TELIGO) which effectively inhibits the *telomerase* enzyme (a ribonucleoprotein *complex*). The advantages of such a strategy include fewer competing sites (there are less than approximately 50 endogenous sites in the *human* genome), inhibition of *telomerase* activity involves both hybridization of complementary sequences of nucleic acids (antisense) and allosteric *binding* (aptameric *binding*) to the ribonucleoprotein *complex* , the telomere which the TELIGO mimics is identical in all higher vertebrates, hence drug development can utilize animal models, and. . .

DETD . . . protection, positioning, and replication. Generally, telomeres consist of hundreds to thousands of tandem repeats of a telomere motif sequence and *associated* proteins. In humans and other *mammals* this motif is 5'-d(TTAGGG)-3'. Sequences specific to other species such as yeasts, plants and ciliates may be found in Greider, Carrol, *Telomeres* *Telomerase* and Senescence", Bio Assays, Vol. 12, No. 8, August 1990, pp. 363-369, incorporated herein by reference.

L15 ANSWER 20 OF 23 USPATFULL

DETD *Human* fetal adrenal gland, ovary, testis, and brain tissues were obtained from normal fetuses at 16 and 18 weeks of gestational. . . died of complications of surgery, and a 5-year old child who had died with CNS abnormalities and congenital heart disease *associated* with Pena-Shokier syndrome. Normal adult testis and other tissues were obtained from a 37-year old male who had died of acute heart failure. All tissues were stored at -80.degree. C. until used. Cell extracts and *telomerase* activity assays were carried out as described in Example 1.

L15 ANSWER 21 OF 23 USPATFULL

SUMM While the methods of the invention are broadly applicable to the detection of *telomerase* activity in any sample from any origin, the methods are especially useful and applicable to the detection of *telomerase* activity in samples of biological material obtained from humans. Such samples will contain cells or cellular materials and will typically be obtained from humans for purposes of detecting cancer. *Telomerase* is not expressed by normal post-natal *human* somatic cells, although low levels of *telomerase* activity can be detected in certain stem cells and activated cells of the hematopoietic system, so the presence of *telomerase* activity in a sample of *human* somatic tissue or cells indicates that immortal cells, including certain types of cancer cells, are present in the tissue. While not all cancer cells express *telomerase* activity, *telomerase* expression is required for cells to become immortal. Consequently, the presence of cells with *telomerase* activity is *associated* with many forms of cancer and can also serve to indicate that a particularly invasive or metastatic form of cancer. . .

DETD This requirement for the *telomerase* substrate to lack telomeric repeat sequences arises out of the second reaction of the present method--the non- *telomerase* -mediated primer extension reaction. In this reaction, an oligonucleotide primer that hybridizes only to extended *telomerase* substrates is added to the reaction mixture under conditions such that, if extended *telomerase* substrates are present, the primer *binds* to the extended substrates and is then extended by enzymatic action. Because *telomerase* can extend the *telomerase* substrate only by the addition of telomeric repeats, the primer will necessarily comprise a sequence complementary to a telomeric repeat. If the *telomerase* substrate employed in the *telomerase* extension reaction comprised a telomeric repeat, then the primer employed in the primer extension reaction could hybridize to unextended *telomerase* substrate, with potentially negative consequences. The *telomerase* substrate can, however, comprise sequences highly related to a telomeric repeat sequence without compromising the validity of the results obtained. For instance, an especially preferred *human* *telomerase* substrate of the invention is oligonucleotide M2, also known as TS (SEQ ID. NO.1), which contains a sequence at its 3'-end that is identical to five of the six bases of the *human* telomeric repeat but otherwise contains no telomeric repeat sequences.

DETD . . . type of any origin and can be used to detect an immortal cell of any origin, provided the cell expresses *telomerase* activity. For *human* samples, the detection of immortal cells will typically be used to detect the presence of cancer cells of any of. . . cell, papillary, scirrhous, bronchiolar, bronchogenic, squamous cell, and transitional cell), histiocytic disorders, leukemia (e.g., B-cell, mixed-cell, null-cell, T-cell, T-cell chronic, HTLV-II- *associated* , lymphocytic acute, lymphocytic chronic, mast-cell, and myeloid), istiocytosis

malignant, Hodgkin's disease, immunoproliferative small, non-Hodgkin's lymphoma, plasmacytoma, eticuloendotheliosis, metanoma, chondroblastoma, chondroma,. . .

DETD The limit of *telomerase* detection in 102 cells was confirmed by TRAP assays of serial dilutions of an extract from 104 293 cells. This. . .] (SEQ ID NO.11) instead of CX (SEQ ID NO.2) further increases sensitivity, although these primers are more likely to *interact* with the unextended TS primer (SEQ ID NO.1). The limit of sensitivity was also analyzed by titration of the synthetic *telomerase* product TS+4 (which contains oligonucleotide TS (SEQ ID NO.1) followed by four telomeric repeats). Dilutions of TS+4 oligonucleotide were mixed with heat-treated (*telomerase* inactivated) 293 extract and analyzed in TRAP assays. In this analysis, the assay gave a clear positive signal from 106 molecules of TS+4. In addition, *telomerase* activity from *mouse* tissue (*telomerase* activity is present in somatic cells of mice) and cell extracts was detected by TRAP assay [redacted]ough the *mouse* *telomerase* by conventional assay was shown to be mostly non-processive (i.e. [redacted] only a single repeat; Prowse et al., 1993, Proc. Natl. Acad. Sci. USA 90:1493-1497), indicating that the TRAP assay is detecting very low levels of processire *mouse* *telomerase* activity that cannot be visualized by the conventional assay.

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SUMM . . . first aspect, the present invention provides the RNA component of, as well as the gene for the RNA component of, *human* *telomerase* in substantially pure form, as well as nucleic acids comprising all or at least a useful portion of the nucleotide sequence of the RNA component of *human* *telomerase* . The present invention also provides RNA component nucleic acids from other species, which nucleic acids share substantial homology with the RNA component of *human* *telomerase* , including but not limited to, the RNA components of *mammals* , such as primates. Other useful nucleic acids of the invention include nucleic acids with sequences complementary to the RNA component; nucleic acids with sequences related to but distinct from nucleotide sequences of the RNA component and which *interact* with the RNA component or the gene for the RNA component or the protein components of *human* *telomerase* in a useful way; and nucleic acids that do not share significant sequence homology or complementarity to the RNA component. . .

SUMM . . . triple helix-forming oligonucleotide, or other oligonucleotide that can be used in vivo or in vitro to inhibit the activity of *human* *telomerase* . Such oligonucleotides can block *telomerase* activity in a number of ways, including by preventing transcription of the *telomerase* gene (for instance, by triple helix formation) or by *binding* to the RNA component of *telomerase* in a manner that prevents a functional ribonucleoprotein *telomerase* from assembling or prevents the RNA component, once assembled into the *telomerase* enzyme *complex* , from serving as a template for telomeric DNA synthesis. Typically, and depending on mode of action, these oligonucleotides of the. . . or more nucleotides that is either identical or complementary to a specific sequence of nucleotides in the RNA component of *telomerase* or the gene for the RNA component of *telomerase* .

SUMM Another type of useful nucleic acid of the invention is a ribozyme able to cleave specifically the RNA component of *human* *telomerase* , rendering the enzyme inactive. Yet another type of useful nucleic acid of the invention is a probe or primer that *binds* specifically to the RNA component of *human* *telomerase* and so can be used, e.g., to detect the presence of *telomerase* in a sample. Finally, useful nucleic acids of the invention include recombinant expression plasmids for producing the nucleic acids of the invention. One especially useful type of such a plasmid is a plasmid used for *human* gene therapy. Useful plasmids of the invention for *human* gene therapy come in a variety of types, including not only those that encode antisense oligonucleotides or ribozymes but also those that drive expression of the RNA component of *human* *telomerase* or a deleted or otherwise altered (mutated) version of the RNA component of *human* (or other species with RNA component sequences substantially homologous to the *human* RNA component) *telomerase* or the gene for the same.

SUMM In a second aspect, the invention provides methods for treating a condition *associated* with the *telomerase* activity within a cell or group of cells by contacting the cell(s) with a therapeutically effective amount of an agent that alters *telomerase* activity in that cell. Such agents include the *telomerase* RNA component-encoding nucleic acids, triple helix-forming oligonucleotides, antisense oligonucleotides, ribozymes, and plasmids for *human* gene therapy described above. In a related aspect, the invention provides pharmaceutical compositions comprising these therapeutic agents together with a. . .

SUMM In a fifth aspect, the invention provides methods for purifying the protein components of *human* *telomerase* as well as the protein components of *telomerase* from a *mammalian* species with an RNA component substantially homologous to the RNA component of *human* *telomerase* . The present invention also provides methods for isolating and identifying nucleic acids encoding such protein components. In related aspects, the present invention provides purified *human* *telomerase* and purified *telomerase* of *mammalian* species with an RNA component substantially homologous to the RNA component of *human* *telomerase* , as well as purified nucleic acids that encode one or more components of such *telomerase* preparations. The present invention also provides pharmaceutical compositions comprising as an active ingredient the protein components of *telomerase* or a nucleic acid that encodes or *interacts* with a nucleic acid that encodes a protein component of *telomerase* .

SUMM The negative selection steps involved the preparation of biotinylated PCR product from cDNA prepared from a *human* cell line that does not have detectable *telomerase* activity. The biotinylated PCR product was denatured and then rehybridized in a solution comprising a much lower concentration of non-biotinylated PCR product (100 biotinylated product: 1 non-biotinylated product) from cDNA prepared from a *human* cell line that does have *telomerase* activity. Given the possibility that the *telomerase* negative cell line might contain some low amount of the RNA component, the hybridization step was conducted to discriminate or. . . to a Co t selected to allow hybridization of the most abundantly expressed RNA, the unwanted material was removed by *binding* to streptavidinylated magnetic particles; the supernatant remaining after particle collection contained the desired cDNA for the RNA component of *human* *telomerase* . The process for PCR amplification of cDNA is described in Example 2, below.

SUMM . . . the invention is an antisense oligonucleotide that can be used in vivo or in vitro to inhibit the activity of *human* *telomerase* . Antisense oligonucleotides comprise a specific sequence of from about 10 to about 25 to 200 or more (i.e., large enough. . . delivery, to administer in vivo, if desired) nucleotides complementary to a specific sequence of nucleotides in the RNA component of *human* *telomerase* . The mechanism of action of such oligonucleotides can involve *binding* of the RNA component either to prevent assembly of the functional ribonucleoprotein *telomerase* or to prevent the RNA component from serving as a template for telomeric DNA synthesis.

SUMM Illustrative antisense oligonucleotides of the invention that serve to inhibit *telomerase* activity in vivo and/or in vitro include the oligonucleotides mentioned above in connection with the tests to determine whether clone pGRN7 comprised the cDNA for the RNA component of *human* *telomerase* . Three such oligonucleotides were synthesized as 2'-O-methyl RNA oligonucleotides, which *bind* more tightly to RNA than DNA oligonucleotides and are more resistant to hydrolysis than unmodified RNA oligonucleotides, and, as noted above, were used to demonstrate inhibition of *telomerase* activity in vitro. The sequence of each of these O-methyl RNA oligonucleotides is shown below. ##STR3## These oligonucleotides can also be used to inhibit *telomerase* activity in *human* cells.

SUMM . . . helix-forming oligonucleotides of the invention, "sense" oligonucleotides identical in sequence to at least a portion of the RNA component of *human* *telomerase* can also be used to inhibit *telomerase* activity. Oligonucleotides of the invention of this type are characterized in comprising either (1) less than the complete sequence of the RNA component needed to form a functional *telomerase* enzyme or (2) the complete sequence of the RNA component needed to form a functional *telomerase* enzyme as well as a substitution or insertion of one or more nucleotides that render the resulting RNA non-functional. In both cases, inhibition of *telomerase* activity is observed due to the "mutant" RNA component *binding* the protein components of *human* *telomerase* to form an inactive *telomerase* molecule. The mechanism of action of such oligonucleotides thus involves the assembly of a non-functional ribonucleoprotein *telomerase* or the prevention of assembly of a functional ribonucleoprotein *telomerase* . Sense oligonucleotides of the invention of this type typically comprise a specific sequence of from about 20, 50 200, 400, 500, or more nucleotides identical to a specific sequence of nucleotides in the RNA component of *human* *telomerase* .

SUMM Other oligonucleotides of the invention called "ribozymes" can also be used to inhibit *telomerase* activity. Unlike the antisense and other oligonucleotides described above, which *bind* to an RNA, a DNA, or a *telomerase* protein component, a ribozyme not only *binds* but also specifically cleaves and thereby potentially inactivates a target RNA, such as the RNA component of *human* *telomerase* . Such a ribozyme can comprise 5'- and 3'-terminal sequences complementary to the *telomerase* RNA. Depending on the site of cleavage, a ribozyme can render the *telomerase* enzyme inactive. See PCT patent publication No.

93/23572, supra. Those in the art upon review of the RNA sequence of the *human* *telomerase* RNA component will note that several useful ribozyme target sites are present and susceptible to cleavage by, for example, a. . . the ribozymes below, which are RNA molecules having the sequences indicated: ##STR4## Other optimum target sites for ribozyme-mediated inhibition of *telomerase* activity can be determined as described by Sullivan et al., PCT patent publication No. 94/02595 and Draper et al., PCT. . .

SUMM Other therapeutic methods of the invention employ the *telomerase* RNA nucleic acid of the invention to stimulate *telomerase* activity and to extend replicative cell life span. These methods can be carried out by delivering to a cell a functional recombinant *telomerase* ribonucleoprotein of the invention to the cell. For instance, the ribonucleoprotein can be delivered to a cell in a liposome, or the gene for the RNA component of *human* *telomerase* (or a recombinant gene with different regulatory elements) can be used in a eukaryotic expression plasmid (with or without sequences coding for the expression of the protein components of *telomerase*) to activate *telomerase* activity in various normal *human* cells that otherwise lack detectable *telomerase* activity due to low levels of expression of the RNA component or a protein component of *telomerase* . If the *telomerase* RNA component is not sufficient to stimulate *telomerase* activity, then the RNA component can be transfected along with genes expressing the protein components of *telomerase* to stimulate *telomerase* activity. Thus, the invention provides methods for treating a condition *associated* with the *telomerase* activity within a cell or group of cells by contacting the cell(s) with a therapeutically effective amount of an agent that alters *telomerase* activity in that cell.

SUMM In related aspects, the invention features pharmaceutical compositions including a therapeutically effective amount of a *telomerase* inhibitor or *telomerase* activator of the invention. Pharmaceutical compositions of *telomerase* inhibitors of the invention include a mutant RNA component of *human* *telomerase* , an antisense oligonucleotide or triple helix-forming oligonucleotide that *binds* the RNA component or the gene for the same of *human* *telomerase* , or a ribozyme able to cleave the RNA component of *human* *telomerase* , or combinations of the same or other pharmaceuticals in a pharmaceutically acceptable carrier or salt. Other pharmaceutical compositions of the invention comprise a *telomerase* activator preparation, such as purified *human* *telomerase* or mRNA for the protein components of *telomerase* and the RNA component of *telomerase* , and are used to treat senescence-related disease. The therapeutic agent can be provided in a formulation suitable for parenteral, nasal,. . .

SUMM In addition, probes or primers that *bind* specifically to the RNA component of *human* *telomerase* (or either strand of the gene for the same) can be used in diagnostic methods to detect the presence of *telomerase* nucleic acid in a sample. Primers and probes are oligonucleotides that are complementary, and so will *bind* , to a target nucleic acid. Although primers and probes can differ in sequence and length, the primary differentiating factor is. . . one of function: primers serve to initiate DNA synthesis, as in PCR amplification, while probes are typically used only to *bind* to a target nucleic acid. Typical lengths for a primer or probe can range from 8 to 20 to 30. . .

SUMM The reagents of the present invention also allow the cloning and isolation of nucleic acids encoding the protein components of *human* as well as other *mammalian* *telomerase* enzymes, which have not previously been available. Access to such nucleic acids provide complementary benefits to those provided by the nucleic acids comprising nucleic acid sequences of the RNA component of *human* *telomerase* . For instance, and as noted above, the therapeutic benefits of the present invention can be enhanced, in some instances, by use of purified preparations of the protein components of *human* *telomerase* and by access to nucleic acids encoding the same. The nucleic acids of the invention that encode the RNA component of *human* *telomerase* can be used to isolate the nucleic acid encoding the protein components of *human* *telomerase* , allowing access to such benefits. Thus, the invention provides methods for isolating and purifying the protein components of *human* *telomerase* , as well as for identifying and isolating nucleic acids encoding the protein components of *human* *telomerase* . In related aspects, the present invention provides purified *human* *telomerase* , purified nucleic acids that encode the protein components of *human* *telomerase* , recombinant expression plasmids for the protein components of *human* *telomerase* . The invention also provides pharmaceutical compositions comprising as an active ingredient either the protein components of *human* *telomerase* or a nucleic acid that either encodes those protein components or *interacts* with nucleic acids that encode those protein components, such as antisense oligonucleotides, triple helix-forming oligonucleotides, ribozymes, or recombinant DNA expression. . .

SUMM The cloned RNA component of *human* *telomerase* can be used to identify and clone nucleic acids encoding the protein components of the ribonucleoprotein *telomerase* enzyme. Several different methods can be employed to achieve identification and cloning of the protein components. For instance, one can. . . enzyme or partially denatured enzyme using as an affinity ligand either (1) nucleotide sequences complementary to the RNA component to *bind* to the RNA component of the intact enzyme; or (2) the RNA component to *bind* the protein components of a partially or fully denatured enzyme. The ligand can be affixed to a solid support or chemically modified (e.g., biotinylated) for subsequent immobilization on the support. Exposure of cell extracts containing *human* *telomerase* , followed by washing and elution of the *telomerase* enzyme bound to the support, provides a highly purified preparation of the *telomerase* enzyme. The protein components can then be optionally purified further or directly analyzed by protein sequencing. The protein sequence determined. . . cloning the cDNA or identifying a clone in a genomic bank comprising nucleic acids that encode a protein component of *telomerase* .

SUMM *Telomerase* RNA *binding* or *telomerase* activity assays for detection of specific *binding* proteins and activity can be used to facilitate the purification of the *telomerase* enzyme and the identification of nucleic acids that encode the protein components of the enzyme. For example, nucleic acids comprising. . . RNA component sequences can be used as affinity reagents to isolate, identify, and purify peptides, proteins or other compounds that *bind* specifically to a sequence contained within the RNA component, such as the protein components of *human* *telomerase* . Several different formats are available, including gel shift, filter *binding* , footprinting, Northwestern (RNA probe of protein blot), and photocrosslinking, to detect such *binding* and isolate the components that *bind* specifically to the RNA component. These assays can be used to identify *binding* proteins, to track purification of *binding* proteins, to characterize the RNA *binding* sites, to determine the molecular size of *binding* proteins, to label proteins for preparative isolation, and for subsequent immunization of animals for antibody generation to obtain antibodies for. . .

CLM What is claimed is:

38. The host cell of claim 37 wherein the recombinant nucleic acid encodes an RNA molecule that can *associate* with protein components of *human* *telomerase* to produce *telomerase* activity capable of adding sequences of repeating units of nucleotides to telomeres.

L15 ANSWER 23 OF 23 USPATFULL

SUMM It has been kown for some years that telomeres in *human* germline cells (e.g. sperm) are longer than those in somatic tissue such as blood. One proposed explanation for this is the absence of telomere repeat addition (i.e. absence of *telomerase* activity) in somatic cells. If so, incomplete end replication would be expected to result in the progressive loss of terminal. . . contribute to the chromosome aberrations typically seen in senescent cells. Senescence and the measurement of cellular time is an intriguingly *complex* subject and it will be interesting to see to what extent telomere shortening has a causal role. The large telomeres. . .

SUMM. . . special structure and behavior of telomeric DNA, suggest that telomere synthesis could be a target for selective drug action. Because *telomerase* activity seems to be essential for protozoans or yeast, but not apparently for *mammalian* somatic cells, I propose that *telomerase* should be explored as a target for drugs against eukaryotic pathogenic or parasitic microorganisms, such as parasitic protozoans or pathogenic yeasts. A drug that *binds* *telomerase* selectively, either through its reverse-transcriptase or DNA substrate-*binding* properties, should selectively act against prolonged maintenance of the dividing lower eukaryote, but not impair the *mammalian* host over the short term, because *telomerase* activity in its somatic cells may normally be low or absent. Obvious classes of drugs to investigate are those directed specifically against reverse transcriptases as opposed to other DNA or RNA polymerases, and drugs that would *bind* telomeric DNA itself. These could include drugs that selectively *bind* the G.G base-paired forms of the G-rich strand protrusions at the chromosome termini, or agents which stabilize an inappropriate G.G. . .

DETD As noted above, the present invention concerns diagnosis and therapy *associated* with measuring telomeric length and manipulating *telomerase* -dependent extension or *telomerase* -independent shortening. While the invention is directed to humans, it may be applied to other animals, particularly *mammals* , such as other primates, and domestic animals, such as equine, bovine, ovine, porcine, feline, and canine. The invention may be. . . may be slowed or inhibited by providing DNA oligonucleotides or their functional equivalent, or self-proliferation can be reduced by inhibiting *telomerase* . In this case of diagnostics, one may detect the length of telomeres as to a particular chromosome or group of chromosomes, or the average length of telomeres. Diagnosis may also be *associated* with determining the activity of *telomerase* in cells, tissue, and the like.

DETD *Telomerase* activity is of interest as a marker of growth potential, particularly as to neoplastic cells, or progenitor cells, e.g., embryonic cells. *Human* *telomerase* activity may be determined by measuring the rate of elongation of an appropriate repetitive sequence (primer), having 2 or more, usually 3 or more, repeats of the telomere unit sequence, TTAGGG. The sequence is labeled with a specific *binding* pair member at a convenient site, e.g., the 5'-terminus, which specific *binding* pair member allows for separation of extended sequences. By using one or more radioactive nucleotide triphosphates or other labeled nucleotide. . . incorporated radioactivity as cpm per unit weight of DNA as a function of unit of time, as a measure of *telomerase* activity. Any other detectable signal and label may also be used, e.g., fluorescein.

skipped refs

AN *1997:740339* CAPLUS DN 128:976
TI identification of new step in telomere maintenance involving S-phase of cell cycle
IN Wellinger, Raymund J.; Zakian, Virginia A.
PA Trustees of Princeton University, USA; Wellinger, Raymund J.; Zakian, Virginia A.
SO PCT Int. Appl., 33 pp. CODEN: PIXXD2 PI WO 9741262 A1 19971106
DS W: CA, US
RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
AI WO 97-US7528 19970502 PRAI US 96-16718 19960502 DT Patent LA English
AB The strand of telomeric DNA that runs 5' to 3' towards a chromosome end is typically G-rich. Due to the properties of conventional DNA polymerases, telomerase-generated tails of this G-rich strand are expected on only one end of individual linear DNA mols. In Saccharomyces, TG1-3 tails are detected on chromosome and linear plasmid telomeres late in S phase. Moreover, the telomeres of linear plasmids can interact when the TG1-3 tails are present. Mols. were generated (in vitro) that mimic the structures predicted for telomere replication intermediates. These (in vivo) generated mols. formed telomere-telomere interactions similar to those on mols. isolated from yeast but only if both ends that interacted had a TG1-3 tail. Moreover, TG1-3 tails were generated (in vivo) in cells lacking telomerase. These data suggest a new step in telomere maintenance, cell cycle regulated degrdn. of the strand which can generate a potential substrate for telomerase and telomere binding proteins at every telomere. The results reported here further suggest that single stranded TG1-3 tails are generated at the end of S-phase on telomerss, irresp. of whether the telomeres were replicated by leading or lagging strand synthesis. These data also suggest that unexpected enzymic activities other than telomerase generate these TG1-3 tails and that their generation is an integral step in telomere maintenance.

AN *1996:702949* CAPLUS DN 126:86441
TI Reconstitution of human telomerase activity and identification of a minimal functional region of the human telomerase RNA
AU Autexier, Chantal; Pruzan, Ronald; Funk, Walter D.; Greider, Carol W.
CS Cold Spring Harbor Lab., Cold Spring Harbor, NY, 11724, USA
SO EMBO J. (1996), 15(21), 5928-5935 CODEN: EMJODG; ISSN: 0261-4189 PB Oxford University Press DT Journal LA English
AB Telomerase is a ribonucleoprotein that catalyzes telomere elongation through the addn. of TTAGGG repeats in humans. Activation of telomerase is often assocd. with immortalization of human cells and cancer. To dissect the human telomerase enzyme mechanism, we developed a functional in vitro reconstitution assay. After removal of the essential 445 nucleotide human telomerase RNA (hTR) by micrococcal nuclease digestion of partially purified human telomerase, the addn. of in vitro transcribed hTR reconstituted telomerase activity. The activity was dependent upon and specific to hTR. Using this assay, truncations at the 5' and 3' ends of hTR identified a functional region of hTR, similar in size to the full-length telomerase RNAs from ciliates. This region is located between positions 1-203. Furthermore, we found that residues 1-44, 5' to the template region (residues 46-56) are not essential for activity, indicating a minimal functional region is located between residues 44-203. Mutagenesis of full-length hTR between residues 170-179, 180-189 or 190-199 almost completely abolished the ability of the hTR to function in the reconstitution of telomerase activity, suggesting that sequences or structures within this 30 nucleotide region are required for activity, perhaps by binding telomerase protein components.

AN 1997:802625 CAPLUS DN 128:139095
TI Characterization and cell cycle regulation of the related human *telomeric* proteins Pin2 and *TRF1* suggest a role in mitosis
AU Shen, Minhui; Haggblom, Candy; Vogt, Marguerite; Hunter, Tony; Lu, Kun Ping
CS Cancer Biol. Program, Div. Hematol./Oncol., Dep. Med., Beth Israel Deaconess Med. Cent., Div. Aging, Harvard med. Sch., Boston, MA, 02215, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1997), 94(25), 13618-13623 CODEN: PNASA6; ISSN: 0027-8424 PB National Academy of Sciences DT Journal LA English
AB *Telomeres* are essential for preserving chromosome integrity during the cell cycle and have been specifically implicated in mitotic progression, but little is known about the signaling mol.(s) involved. The human *telomeric* repeat binding factor protein (***TRF1***) is shown to be important in regulating *telomere* length. However, nothing is known about its function and regulation during the cell cycle. The sequence of PIN2, one of three human genes (PIN1-3) the authors previously cloned whose products interact with the Aspergillus NIMA cell cycle regulatory protein kinase, reveals that it encodes a protein that is identical in sequence to *TRF1* apart from an internal deletion of 20 amino acids; Pin2 and *TRF1* may be derived from the same gene, PIN2/***TRF1*** . However, in the cell Pin2 was found to be the major expressed product and to form homo- and heterodimers with *TRF1*** ; both dimers were localized at *telomeres*** . Pin2 directly bound the human *telomeric* repeat DNA in vitro, and was localized to all *telomeres* uniformly in *telomerase*** -pos. cells. In contrast, in several cell lines that contain barely detectable *telomerase* activity, Pin2 was highly concd. at only a few *telomeres*** . Interestingly, the protein level of Pin2 was highly regulated during the cell cycle, being strikingly increased in G2+M and decreased in G1 cells. Moreover, overexpression of Pin2 resulted in an accumulation of HeLa cells in G2+M. These results indicate that Pin2 is the major human *telomeric* protein and is highly regulated during the cell cycle, with a possible role in mitosis. The results also suggest that Pin2/***TRF1* may connect mitotic control to the *telomere* regulatory machinery whose deregulation has been implicated in cancer and aging.

AN 1997:624987 CAPLUS DN 127:304743
TI Characterization of *human* *telomerase*** *complex***
AU Ramakrishnan, Shyam; Sharma, Harsh W.; Farris, A. Darise; Kaufman, M.; Harley, John B.; Collins, Kathleen; Pruijn, Ger J. M.; Van Venrooij, Walther J.; Martin, Mitchell L.; Nara[illegible]n, Ramaswamy
CS Department of Oncology, Hoffmann-La Roche, Nutley, NJ, 07110, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1997), 94(19), 10075-10079 CODEN: PNASA6; ISSN: 0027-8424 PB National Academy of Sciences DT Journal LA English
AB Telomerase, a ribonucleoprotein complex, adds hexameric repeats called *telomeres* to the growing ends of chromosomal DNA. Characterization of mammalian telomerase has been elusive because of its low level of expression. We describe a bioinformatics approach to enrich and characterize the *human* *telomerase*** *complex*** . Using local sequence homol. search methods, we detected similarity of the Tetrahymena p80 subunit of telomerase with the autoantigen Ro60. Antibodies to Ro60 immunopptd. The telomerase activity. Ro60 and p80 proteins were cross-recognizable by antibodies to either protein. Telomerase activity and the RNA component of telomerase complex were localized to a doublet in a native gel from the Ro60 antibody-pptd. material. The enriched material showed specific binding to a TTA GGG probe in vitro in an RNA template-dependent manner. Polyclonal antibodies to the doublet also immunopptd. the telomerase activity. These results suggest an evolutionary conservation of the telomerase proteins.

AN 1997:70566 CAPLUS DN 126:153192
TI *TRF1*** , a mammalian *telomeric* protein
AU Smith, Susan; De Lange, Titia
CS Rockefeller Univ., New York, NY, 10021, USA
SO Trends Genet. (1997), 13(1), 21-26 CODEN: TRGEE2; ISSN: 0168-9525 PB Elsevier DT Journal; General Review LA English
AB A review with 44 refs. *Telomerase* adds TTAGGG repeats onto mammalian chromosome ends, replenishing the terminal sequence loss incurred during DNA replication. This maintenance of *telomeric* DNA preserves binding sites for *telomeric*** proteins, which form a protective nucleoprotein complex at chromosome ends. The recent isolation of *TRF1*** , the mammalian *telomeric*** -repeat binding factor, should now allow the structure and function of the *telomeric* complex to be examd. in detail.

AN 1996:658943 CAPLUS DN 125:318929
TI A review of the structure and function of *human*** *telomere***
AU Ishikawa, Fuyuki
CS Dep. Life Sci., Tokyo Inst. Technol., Yokohama, 226, Japan
SO Tanpakushitsu Kakusan Koso (1996), 41(15), 2241-2253 CODEN: TAKKAJ; ISSN: 0039-9450 DT Journal; General Review LA Japanese
AB A review with 52 refs., on the structure of human telomere (telomeric repeats and sub-telomeric regions), telomere-binding proteins, localization of human telomere in nucleus, telomere length and telomerase, shortening of telomere and genome destabilization in tumor cells, and roles of homologous recombination in telomeric regions in evolution of chromosomes and species. Functions of telomere and telomerase in cell aging and tumorigenesis are discussed.